[*The Editors of the Biochemical Journal accept no responsibility for the Reports of the Proceedings of the Society. Abstracts of papers read are published as received from the authors.*]

PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **345th Meeting** *of the Biochemical Society was held in the Middlesex Hospital Medical School, Mortimer Street, London, W.* 1, *on* 19*th November* 1955, *at* 11 *a.m., when the following papers were read:*

COMMUNICATIONS

A Simple Gradient Elution Device Applied to the Separation of 17-Oxosteroids. By A. E. Kellie and A. P. Wade. (*Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, W.*1)

The application of the gradient elution principle to the chromatographic separation of urinary oxosteroids has been described by Lakshmanan & Lieberman (1953, 1954), who drew attention to the substantial advantage obtained by the elimination of 'tailing' of adsorbed compounds. In place of the complex apparatus used by these authors, a simple device has been introduced (Kellie, 1953) which has the advantage of cheapness and versatility, and which separates 17-oxosteroids more completely than other non-gradient column methods so far described. Alumina is used as the adsorbent and the column is prepared in the customary manner; the gradient is applied to the column from twin reservoirs, a donor and a recipient, connected by a capillary syphon. The column is fed from the recipient reservoir which initially contains 0·2% (v/v) ethanol in benzene but which receives progressive enrichment from the donor vessel containing 2·0% (v/v) ethanol in benzene. The contents of the recipient reservoir are stirred.

Many factors influence the behaviour of the compounds on the column, viz. the percentage of ethanol in the donor and recipient reservoirs, the dimensions of the column, the flow rate, the weight and moisture content of the alumina, etc., but of particular interest is the ratio of the areas of cross-section of the donor and recipient vessels. By varying this ratio the shape of the gradient curve may be made convex, linear or concave and suited to the particular requirements of the mixture analysed.

A system of concave gradient analysis has been evolved which completely separates 3β-hydroxyandrost-5-en-17-one, 3α-hydroxy-5α-androstan-17-one, 3α-hydroxy-5β-androstan-17-one, 3α-hydroxy-5β-androstan-11:17-dione, 3α:11β-dihydroxy-5α-androstan-17-one and 3α:11β-dihydroxy-5β-androstan-17-one and this has been applied on a microscale for the quantitative analysis of urinary steroids from glucuronide and sulphate fractions. The former were hydrolysed by a β-glucuronidase preparation from the limpet (*Patella vulgaris*) (Dodgson & Spencer, 1953) and the latter by continuous extraction with ether at pH 1. Such analyses can be very laborious and time-consuming, and the present method has been designed to eliminate much of the tedious manual work. The identity and purity of the compounds isolated has been confirmed by paper chromatography and by comparison of the infra-red absorption spectra with those of standard reference compounds.

REFERENCES

Dodgson, K. S. & Spencer, B. (1953). *Biochem. J.* **43**, 99.
Kellie, A. E. (1953). *Ann. Rep. Brit. Empire Cancer Campaign*, **31**, 397.
Lakshmanan, T. K. & Lieberman, S. (1953). *Arch. Biochem. Biophys.* **45**, 235.
Lakshmanan, T. K. & Lieberman, S. (1954). *Arch. Biochem. Biophys.* **53**, 258.

Urinary 17-Oxosteroid Conjugates. Separation of Glucuronides from Sulphates and Further Purification of these Fractions. By A. E. Kellie and A. P. Wade. (*Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, W.* 1)

Urinary 17-oxosteroid conjugates consist of a mixture of glucuronides and sulphates. In the absence of a method for the isolation of individual components of the mixture it has been customary to hydrolyse these conjugates before further analysis. In the present work an attempt is made to analyse the conjugates without hydrolysis. To investigate each class of conjugates independently it is essential to obtain each group free from the other. This has been achieved by obtaining the conjugates in a dry form (Edwards, Kellie & Wade, 1953) and acetylating the mixture. Of the 17-oxosteroid conjugates known to be present, only the glucuronides have hydroxyl groups which can be acetylated and this acetylation converts these conjugates from water-soluble benzene-insoluble compounds to water-insoluble benzene-soluble compounds. Acetylated glucuronides can therefore

be separated from the sulphates, which remain soluble in water. The efficiency and correctness of the procedure has been tested by comparing the 17-oxosteroids present in glucuronide and sulphate fractions prepared in this way, with the 17-oxosteroids present in corresponding fractions obtained by enzymic hydrolysis of the glucuronides. The sulphates were hydrolysed by acidifying an aqueous solution and extracting continuously with ether for 72 hr.

In a further investigation of the glucuronide fraction the conjugates were methylated with diazomethane before acetylation. After this procedure it was possible to separate quantitatively the ketonic from the non-ketonic glucuronides by a conventional Girard separation. A considerably purified ketonic fraction was obtained which could be estimated by the Zimmermann reaction for 17-oxosteroids without preliminary hydrolysis. Chromatography of this ketonic fraction on alumina using a gradient elution system gave separation into four or five components, two of which have been shown by hydrolysis and paper chromatography to contain either 3α-hydroxy-5α-androstan-17-one or 3α-hydroxy-5β-androstan-17-one.

The sulphate fraction was purified by extracting an aqueous solution continuously with ether at neutral pH followed by continuous extraction with chloroform. Chromatography of the chloroform extract on alumina and elution with benzene containing an increasing concentration of methanol, gave fractions on which the Zimmermann reaction could be performed without preliminary hydrolysis.

REFERENCE

Edwards, R. W. H., Kellie, A. E. & Wade, A. P. (1953). *Mem. Soc. Endocrin.* **2**, 53.

Chemical Changes Occurring during Electrolytic Desalting. By I. Smith, B. J. Stevens and J. B. Jepson. (*Courtauld Institute of Biochemistry, Middlesex Hospital, W.* 1)

Previous studies of a significant chemical reaction occurring during the process of electrolytic desalting have been concerned with the conversion of arginine to ornithine (Stein & Moore, 1951; Astrup, Stage & Olsen, 1951). Apart from this, it is often assumed that organic molecules remain relatively untouched while inorganic ions are rapidly discharged and removed. This is far from being the case. Although the arginine conversion can be brought to negligible proportions by a suitably designed apparatus, many other chemical and physical changes can be induced by the process, and the true interpretation of chromatograms from 'desalted' solutions depends on the recognition or anticipation of these changes. The course and rate of reactions occurring during desalting have been studied by serial paper chromatograms.

Although there is no loss of weak organic acids (Katz & Chaikoff, 1954), strong organic anions, e.g. sulphate conjugates in urine, pass rapidly through the membrane and can be recovered from the effluent anodic acid. Tyrosine may precipitate from a completely 'desalted' solution and so may be missed.

During desalting, reductive dehalogenation of substituted phenols occurs, most readily with iodo-, least readily with chloro-, and not at all with fluoro-derivatives. Poly-iodo- and poly-bromo-derivatives of tyrosine and thyronine are dehalogenated in a stepwise manner, and the numerous products can be separated chromatographically.

Other reductive reactions include the general conversion of substituted acrylic acids to the corresponding propionic acids (the case of urocanic acid was described by Zenisek & Kral, 1953), and the hydrogenation of pyridine carboxylic acids to ninhydrin-positive piperidine and dehydro-piperidine derivatives, including compounds of the baikiain type.

The chemical changes consequent on electrolytic desalting in no wise condemn the use of the method. Indeed, the identification of a substance may be facilitated by its behaviour on desalting, and the apparatus has also proved a useful preparative tool, e.g. for making monobromo- and monoiodo-tyrosine.

REFERENCES

Astrup, T., Stage, A. & Olsen, E. (1951). *Acta chem. scand.* **5**, 1343.

Katz, J. & Chaikoff, I. L. (1954). *J. biol. Chem.* **206**, 887.

Stein, W. H. & Moore, S. (1951). *J. biol. Chem.* **190**, 103.

Zenisek, A. & Kral, J. A. (1953). *Biochim. biophys. Acta*, **12**, 479.

Endogenous Respiration and Glycogen Reserves in Yeast. By L. H. STICKLAND. (*Department of Experimental Pathology and Cancer Research, School of Medicine, Leeds*)

The nature of the substrate of the endogenous respiration of yeast is disputed; Meyerhof (1925) found the R.Q. of yeast respiring without added substrate was 0·85, but Stier & Stannard (1936) and Spiegelman & Nozawa (1945) with a number of varieties of yeast found a value of 1·00 and deduced a polysaccharide nature for the substrate.

Yeast without added substrate shows no fermentation (Stier & Stannard, 1936), but the addition of dinitrophenol (DNP) anaerobically induces a fermentation, largely at the expense of stored carbohydrate (Rothstein & Berke, 1952).

In the present experiments, with ordinary baker's yeast, the R.Q. was 0·85, and during the respiration no polysaccharide disappeared from the yeast (cf. Fürth & Lieben, 1922). On the addition of DNP aerobically, the amount of respiration was roughly unaltered, an aerobic fermentation was set up, and the amount of polysaccharide disappearing corresponded to the sum of the oxygen consumption and the extra carbon dioxide production, on the assumption that stored carbohydrate was the sole substrate of both respiration and fermentation.

The conclusion is that in normal yeast the stored carbohydrate is not available for use either aerobically or anaerobically, but DNP causes a complete switch-over to carbohydrate breakdown. No explanation of the differences of R.Q. observed by different investigators can be offered.

REFERENCES

Fürth, O. & Lieben, F. (1922). *Biochem. Z.* **128**, 144.
Meyerhof, O. (1925). *Biochem. Z.* **162**, 43.
Rothstein, A. & Berke, H. (1952). *Arch. Biochem. Biophys.* **36**, 195.
Spiegelman, S. & Nozawa, M. (1945). *Arch. Biochem.* **6**, 303.
Stier, T. J. B. & Stannard, J. N. (1936). *J. gen. Physiol.* **19**, 479.

Stability of Tryptophan during the Alkaline Hydrolysis of Proteins. By A. DRÈZE (introduced by W. S. REITH). (*Department of Biochemistry, Faculty of Medicine, University of Brussels*)

The method of Moore & Stein (1949) has been used for the quantitative determination of tryptophan under various conditions of protein hydrolysis. It was found that there was a considerable destruction when tryptophan (0·01 mM) was heated in a sealed glass tube (20 hr., 120°) in NaOH (5N, 5 ml.). This destruction could be prevented by the addition of a reducing agent such as stannous chloride (5%, w/v). In the presence of starch, the recovery of tryptophan was found to be inversely proportional to the quantity of carbohydrate contained in the solution. If, however, a more dilute solution was used (0·01 mM tryptophan, in 15–25 ml. 6N-$Ba(OH)_2$), addition of starch, even up to 0·5 g., did not cause any destruction.

Tryptophan destruction by cystine (0·04 mM), serine (0·15 mM) and threonine (0·12 mM) under similar experimental conditions was also examined. In the absence of carbohydrate the recoveries were low, but an addition of starch (0·5 g.) and the use of dilute solutions prevented tryptophan destruction.

The protective action of starch was further studied by heating a mixture of *N*-acetyl-DL-tryptophan (0·01 mM), bovine serum albumin hydrolysate (160 mg.) and starch (500 mg.) with 6N-$Ba(OH)_2$ (15 ml.) in a sealed glass tube (15 hr., 120°). Under these conditions tryptophan was quantitatively recovered.

Finally, the hydrolytic conditions generally used (100 mg. protein, 5 ml. 5N-NaOH) were compared with those now found to be more suitable (100 mg. protein, 15 ml. 6N-$Ba(OH)_2$, 500 mg. starch). In both cases bovine serum albumin was used. The yield of tryptophan under normal conditions was 0·48%, and under our conditions was 0·63%.

I thank Professor E. J. Bigwood and Dr S. Moore for their kind interest and encouragement throughout this work, which was carried out while I was a Chargé de Recherches du Fond National de la Recherche Scientifique.

REFERENCE

Moore, S. & Stein, W. H. (1949). *J. biol. Chem.* **178**, 53.

'Pain-Producing Substance' and Human Bradykinin. By J. B. JEPSON, D. ARMSTRONG, C. A. KEELE and J. W. STEWART. (*Middlesex Hospital Medical School, W.* 1)

Fresh human blood plasma which has never been in contact with glass has no action in contracting the isolated rat uterus, nor does it cause pain when applied to the area of an opened skin blister. Such plasma stored in polythene maintains this inactive state for several hours. If, however, it is put in contact with glass, uterine-stimulating and pain-producing activity rapidly develops; maximum activity is attained after about 5 min. in glass, but the activity then decays, 90% disappearing in

30 min. We define this activity as due to 'pain-producing substance' (PPS), without implying that a single molecular species is responsible (Armstrong, Keele, Jepson & Stewart, 1954).

Precipitation of activated plasma by boiling ethanol gives a protein-free alcoholic extract containing the active principle in stable form. Chemical and enzymic evidence suggests that PPS is a peptide material split from a larger molecule by a process induced by glass contact.

In its pharmacological reactions, PPS is very similar to the peptide bradykinin (Roche e Silva, 1955*a*), generated from bovine plasma α_2-globulin by snake venom or trypsin. The most useful substrate for bradykinin production by trypsin is made by the acetic acid denaturation of the total plasma proteins [a 'van Arman' substrate (van Arman, 1955)]. A preparation of human bradykinin has been made in this way, and in its pharmacological behaviour it could not be distinguished from bovine bradykinin or from PPS extracted by ethanol from glass-activated human plasma. A comparison of the effect of proteolytic enzymes on PPS and bradykinin has been made, an approach successfully used to distinguish wasp-venom kinin from bovine bradykinin (Schachter & Thain, 1954; Roche e Silva, 1955*b*).

The nature of the glass-induced process which causes the appearance of PPS in human plasma is unknown. But one thing is clear—the protein *mélange* of blood investigated in glass apparatus differs in some way, however subtle, from that of blood maintained in polythene, and presumably from that *in vivo*.

We thank Dr M. Roche e Silva for his help and advice in some of this work.

REFERENCES

van Arman, C. G. (1955). In *Polypeptides which Stimulate Plain Muscle*, ed. J. H. Gaddum, p. 103. Edinburgh.

Armstrong, D., Keele, C. A., Jepson, J. B. & Stewart, J. W. (1954). *Nature, Lond.*, **174**, 791.

Roche e Silva, M. (1955*a*). In *Polypeptides which Stimulate Plain Muscle*, ed. J. H. Gaddum, p. 45. Edinburgh.

Roche e Silva, M. (1955*b*). *Proc. Int. Conf. on Animal Venoms*, p. 26. (California, Jan. 1955), Amer. Ass. Adv. Sc.

Schachter, M. & Thain, E. M. (1954). *Brit. J. Pharmacol.* **9**, 352.

The Effects of Chylomicra and of Ethanolamine Phosphatide on the Generation of Thrombin during Blood Coagulation. By D. S. Robinson and J. C. F. Poole. (*Sir William Dunn School of Pathology, University of Oxford*)

Poole (1955) showed that when most of the particulate fat was removed from human plasma by centrifugation at high speeds the coagulation time of the resulting infranatant plasma on recalcification was markedly lengthened. The defect could be corrected by adding the fat particles recovered by the centrifugation procedure, or by adding chyle obtained by cannulating the thoracic duct of a rat which had been fed olive oil.

Robinson (1955) showed that the fat particles from rat chyle (chylomicra), when washed by repeated centrifuging so as to be free of water-soluble lymph components, contained, besides triglycerides, small amounts of phosphatide and cholesterol but no measurable amount of protein.

Since adding triglyceride or cholesterol to plasma has no effect on coagulation it seemed possible that the observed coagulant activity of chylomicra was due to a phosphatide.

The action of rat chylomicra on coagulation has been compared with that of a number of purified phosphatides. Adding rat chylomicra to plasma from which most of the particulate fat has been removed by high speed centrifugation greatly increases the amount of thrombin formed in a thrombin generation test. Several ethanolamine phosphatide preparations also increase the amount of thrombin generated in similar tests whereas lecithin, serine phosphatide and inositol phosphatide are inactive.

Acid hydrolysates of chyle contain a substance resembling ethanolamine in its ionophoretic and chromatographic behaviour. Substances resembling choline and serine are also present.

It is thought that ethanolamine phosphatide is probably the active substance in the chylomicron and the possibility that it may play a more general role in blood coagulation must be considered.

A fuller account of this work is being published in the *Quarterly Journal of Experimental Physiology*. It was carried out during the tenure of the Alan Johnston, Lawrence and Moseley Research Fellowship of the Royal Society by D. S. Robinson, and of the Locke Research Fellowship of the Royal Society by J. C. F. Poole.

REFERENCES

Poole, J. C. F. (1955). *Brit. J. Haematol.* **1**, 229.

Robinson, D. S. (1955). *Quart. J. exp. Physiol.* **40**, 112.

The Hydrolysis of Substituted Phenyl Sulphates by the Arylsulphatase of *Alcaligenes metalcaligenes*. By K. S. Dodgson, B. Spencer and K. Williams. (*Department of Biochemistry, University College, University of Wales, Cardiff*)

Consideration of the effect of pH on the substrate affinity of the arylsulphatase of *Alcaligenes metalcaligenes* acting on *p*-nitrophenyl-, *p*-acetylphenyl- and 2-hydroxy-5-nitrophenyl sulphates (Dodgson, Spencer & Williams, 1955) indicated that both positively and negatively charged ionizing groups were present at the active site of the enzyme. An electrostatic attraction between these groups and oppositely charged centres in the substrate molecules was proposed to explain enzyme–substrate interaction. Further information on the mode of action of the enzyme has now been obtained by considering the effect of substitution in the benzene ring on the kinetics of the hydrolysis of phenyl sulphate (cf. Gawron, Grelecki & Duggan, 1953; Nath & Rydon, 1954).

The Michaelis constants (K_m) and maximum velocities ($v_{max.}$) for a number of substituted phenyl sulphates were determined by the method of Lineweaver & Burk (1934) and these values were then plotted logarithmically against the appropriate substitution constants (σ) (Hammett, 1940). The affinity of the enzyme for substrate ($1/K_m$) and the maximum velocity were markedly affected by substitution and progressively increased with the increasing electrophilic nature of the substituent group. When the substituent groups were nucleophilic in nature (e.g. OH and NH_2) the degree of hydrolysis was very low and was difficult to measure accurately under the standard experimental conditions. The withdrawal of electrons from the ester sulphate side chain would thus seem to be an important pre-requisite for arylsulphatase activity. The possible mode of action of the *Alcaligenes* enzymes will be discussed in the light of these and earlier findings.

There are grounds for suspecting that the various arylsulphatases which occur in nature may not necessarily have a common mode of action. However, it seems likely that the inability of the arylsulphatase of *Aspergillus oryzae* (Takadiastase) to hydrolyse *o*-aminoarylsulphates (Boyland, Manson, Sims & Williams, 1955) indicates that a mode of action similar to that of the *Alcaligenes* enzyme is involved in this case also.

REFERENCES

Boyland, E., Manson, D., Sims, P. & Williams, D. C. (1955). *Biochem. J.* **59**, xix.

Dodgson, K. S., Spencer, B. & Williams, K. (1955). *Biochem. J.* (in the Press).

Gawron, O., Grelecki, C. J. & Duggan, M. (1953). *Arch. Biochem.* **44**, 455.

Hammett, L. P. (1940). *Physical Organic Chemistry*, p. 184. New York: McGraw-Hill.

Lineweaver, H. & Burk, D. (1934). *J. Amer. chem. Soc.* **56**, 568.

Nath, R. L. & Rydon, H. N. (1954). *Biochem. J.* **57**, 1.

Paper Chromatography of Hydrolysis Products of Phospholipids. By J. Olley. (*Torry Research Station, Aberdeen*) and R. M. C. Dawson (*Biochemistry Department, Institute of Animal Physiology, Babraham*)

Paper chromatography and electrophoresis are increasingly being used to identify and estimate phosphate esters present in tissues as the free metabolites, and in phospholipid hydrolysates (Rouser, Berry, Marinetti & Stotz, 1953; Huennekens, Hanahan & Uzeil, 1954; Dawson, 1954; Olley, 1955; Wade & Morgan, 1955). With certain phosphate esters, important in lecithin metabolism and also found in phospholipid hydrolysates, we have observed serious discrepancies between the R_F values quoted in the literature (Rouser *et al.* 1953; Huennekens *et al.* 1954) and those obtained independently in our own laboratories. It is considered that these are beyond the range of small variations to be expected when R_F values are determined in different laboratories.

Phosphorylcholine (PC) was synthesized by the method of Plimmer & Burch (1937). Another synthetic sample was kindly supplied by Dr E. Baer, and an enzymically prepared specimen was a gift from Dr M. Macfarlane. Analytically pure L-α-glycerylphosphorylcholine (GPC) was prepared from both ovolecithin and synthetic distearoyl-lecithin (Dawson, 1954), and a synthetic sample was kindly provided by Dr D. Clouet. R_F values for PC, GPC, β-glycerophosphoric acid (GPA) and choline chloride, using various solvent systems with acid-washed Whatman no. 1 paper were as follows: *t*-butanol–picric acid–H_2O (80:4:20, v/w/v); PC 0·48, GPC 0·27, GPA 0·64: phenol saturated with water; PC 0·87, GPC 0·90, GPA 0·34: *n*-propanol–acetic acid–H_2O (8:1:1, v/v/v); PC 0·08, GPC 0·09, GPA 0·12, choline 0·24: *n*-propanol–H_2O (8:1); PC 0·00, GPC 0·04, GPA 0·02, choline 0·2: ethanol–H_2O (8:1); PC 0·10, GPC 0·25, GPA 0·13, choline 0·43. The chromato-

grams were developed at room temperature; little variation in R_F was caused by equilibration time, ascending or descending chromatography, washed or unwashed papers, pretreatment with versene, texture of paper, or whether the phosphate esters were applied as free acids or salts.

It would therefore appear that some unknown factor can cause a very marked variation in the rate of running of these and probably other phosphate esters on chromatograms developed under apparently identical experimental conditions.

When sphingomyelin was hydrolysed in methanolic HCl (1·4N HCl for 1·5 hr. in a sealed tube and 6N under reflux for 6 hr.) chromatographic examination showed that phosphorylcholine but no free choline had been produced. On paper chromatography of the aqueous soluble fraction of the hydrolysate in *n*-butanol–acetic acid–H_2O (4:1:5), the phosphorylcholine (R_F 0·1) could be separated from two ninhydrin-reacting compounds (R_F's 0·65, 0·88).

REFERENCES

Dawson, R. M. C. (1954). *Biochim. biophys. Acta.* **14**, 374.

Huennekens, F. M., Hanahan, D. J. & Uzeil, M. (1954). *J. biol. chem.* **206**, 443.

Olley, J. (1955). *2nd International Conference on Biochemical Problems of Lipids* (in the Press).

Plimmer, R. H. A. & Burch, W. J. N. (1937). *Biochem. J.* **31**, 398.

Rouser, G., Berry, J. F., Marinetti, G. & Stotz, E. (1953). *J. Amer. chem. Soc.* **75**, 310.

Wade, H. E. & Morgan, D. M. (1955). *Biochem. J.* **60**, 264.

Synthesis of Crystallizable Porphobilinogen. By J. J. Scott.* (*National Institute for Medical Research, Mill Hill, London, N.W.* 7)

The structure of porphobilinogen (PBG) has been determined by Cookson & Rimington (1954). PBG is also known to be an intermediate in porphyrin biosynthesis (Falk, Dresel & Rimington, 1953). An enzyme, aminolaevulic dehydrase, which catalyses the unsymmetrical condensation of δ-aminolaevulic acid (ALA) specifically, to give PBG, has recently been isolated (Gibson, Neuberger & Scott, 1954; Granick, 1954; Schmid & Shemin, 1955). Experiments will be described which not only show that ALA may be converted to PBG by chemical means, but also indicate that the reaction may be general for α-aminoketones, leading in each case to an α-aminomethylpyrrole.

For studies of ALA metabolism (Berlin, Neuberger & Scott, 1954), it was necessary to inject strong neutralized solutions of ALA hydrochloride. A few hours after mixing, such solutions would give a magenta colour with Ehrlich's reagent; a similar observation was made by Shemin (1955). The compound responsible for production of this colour has proved to be PBG. From the initial collaborative observations, the following general procedure for PBG synthesis has been elaborated.

Alkali is added to ALA hydrochloride *in vacuo*. After several days at 18°, air is readmitted and PBG isolated from the reaction mixture and crystallized by a modification of the method (Cookson & Rimington, 1954) for isolating PBG from urine. The best yield so far, based on crystalline PBG, has been 3%. This is believed to be the first synthetic PBG isolated. Enzymic synthesis gives greatly superior yields, but cannot be extended to other α-aminomethylpyrroles. By the method outlined above other α-amino-ketones have been condensed to pyrroles in an analogous manner. Effects of pH and temperature on the reaction are complex, and will be discussed.

The presence of PBG in mixtures derived from other newly synthesized pyrroles (Prasad & Raper, 1955) has been demonstrated chromatographically (Rimington & Krol, 1955) but no PBG was isolated. The crystalline material obtained in the present study is indistinguishable from PBG of biological origin chromatographically, also by comparison of X-ray powder diagrams, and by measurement of the extinction coefficient of the *p*-dimethylaminobenzaldehyde adduct. Very recently MacDonald (1955) has completed a 16-stage synthesis of PBG along more conventional lines, with an overall yield of 0·5%.

I am grateful to Mrs O. Kennard for the X-ray photography.

REFERENCES

Berlin, N. I., Neuberger, A. & Scott, J. J. (1954). Vth International Congress of Haematology, Paris, *Abstracts*, p. 279.

Cookson, G. H. & Rimington, C. (1954). *Biochem. J.* **57**, 476.

Falk, J. E., Dresel, E. I. B. & Rimington, C. (1953). *Nature, Lond.*, **172**, 292.

Gibson, K. D., Neuberger, A. & Scott, J. J. (1954). *Biochem. J.* **58**, xli.

Granick, S. (1954). *Science*, **120**, 1105.

MacDonald, S. F. (1955). Personal communication.

Prasad, K. S. N. & Raper, R. (1955). *Nature, Lond.*, **175**, 629.

Rimington, C. & Krol, S. (1955). *Nature, Lond.*, **175**, 629.

Schmid, R. & Shemin, D. (1955). *J. Amer. chem. Soc.* **77**, 506.

Shemin, D. (1955). Personal communication.

* Present address: Department of Chemical Pathology, St Mary's Hospital Medical School, London, W. 2.

The Valine/Leucine Decarboxylase of *Proteus vulgaris*; a Study in Coenzyme Dissociation.
By L. Ekladius and H. K. King. (*Department of Biochemistry, University of Liverpool*)

Proteus can decarboxylate valine, leucine and certain other amino acids (King, 1953) through a single enzyme system requiring pyridoxal phosphate. With valine, the decarboxylation follows a linear course over a period of at least 30 min. from addition of the substrate. Leucine gives an initial rate of decarboxylation about equal to that of valine, but after the first few minutes the reaction slows considerably. If pyridoxal or pyridoxal phosphate is added, leucine will maintain its initial rate of decarboxylation. The decarboxylation of valine is not accelerated by addition of the coenzyme. Cell-free extracts (prepared by grinding with alumina, or by shaking with ballotini) decarboxylate valine slowly, and are inactive towards leucine; activity towards both amino acids is restored completely on adding pyridoxal phosphate (but not pyridoxal). The amount of coenzyme required is larger in the case of leucine, the K_s values (half-maximal velocity) being $0{\cdot}3 \times 10^{-6}$M for valine and 5×10^{-6}M for leucine. This difference in affinity is reflected in the greater sensitivity of leucine decarboxylation towards inhibition by the carbonyl fixatives, hydroxylamine and semicarbazide; though the sensitivity of the enzyme towards a wide range of other inhibitors did not depend on the substrate.

It seems that the particular substrate being metabolized may influence the affinity of the enzyme for its coenzyme. This does not explain, however, why the *initial* rate of decarboxylation of leucine (in the absence of added coenzyme) should be equal to the rate maintained under conditions of coenzyme saturation. The reaction with leucine may perhaps loosen the bond between enzyme and coenzyme. This is suggested by experiments in which leucine is added to suspensions of the organism which are already decarboxylating valine; the overall rate of decarboxylation falls, over a period of several minutes, to the slower rate characteristic of leucine. Neither enzyme nor coenzyme are inactivated by interaction with leucine, nor are the products of leucine decarboxylation inhibitory.

REFERENCE

King, H. K. (1953). *Biochem. J.* **54**, xi.

Free Sugar Estimation in Codling (*Gadus callarias*) Muscle. By N. R. Jones. (*Torry Research Station, Food Investigation Organisation, Department of Scientific and Industrial Research, Aberdeen*)

Reductometric procedures are unreliable for codling muscle extracts of high free amino acid content and are also non-specific. Direct quantitative paper chromatography is satisfactory for some species with high ribose contents (Tarr, 1954). With many codling extracts this procedure gave distortion of chromatograms by 'salt' and low recoveries of added ribose. Freeze-dried, chloroform-separated 75% (v/v) ethanol extracts and electrolytically desalted ether-extracted trichloroacetic acid extracts also gave low recoveries, resulting from 'browning' reactions with amino acid during concentration and application at the start-line. Amino acid composition controlling such losses varies with the season and freshness. Recoveries of added glucose were satisfactory.

Removal of interfering salts and acids by ion-exchange resin mixtures containing strong-base resin (OH) lowers glucose recovery (Hulme, 1953). Dowex 2 (CO_3) plus IR-120 (H) failed to remove taurine from ether-extracted, trichloroacetic acid extracts. This precluded reductometric analysis, but taurine has not interfered with chromatographic determination. A mixture of 2 g. 1:1 IR-120 (H): IR-4B (OH) (Strange, Dark & Ness, 1955) to separated ethanol extracts or ether-extracted trichloroacetic acid extracts equivalent to 4–10 g. muscle was equally effective in removing acids other than taurine, and without gas-evolution difficulties.

Batch-shaking periods above 15 min. may give increasing apparent free ribose levels resulting from adenosine hydrolysis by IR-120 (H). No measurable free ribose was produced from bound ribose when orcinol-reacting substances were shaken for 15 min. with resin at extract concentrations. Glucose was not liberated from bound forms and values agreed closely with those for untreated extracts.

Using the latter resin procedure, chromatography in butanol-acetic acid, and spot elution (Baar, 1954), 9–36 mg. glucose/100 g. were determined in muscle from freshly killed line-caught or trawled codling. After 5 days' storage in ice under fish trade conditions levels fell reaching 0–6 mg./100 g. after 19 days. Ribose increased from 0 to 3·6–4·0 mg. at 12 days and thereafter fell. In most cases, sterile muscle blocks stored at 0° C. (Hodgkiss & Jones, 1955) gave only trace glucose values after 5 days: ribose increased to 3·8–5·2 mg. after

12 days and 6·3–7·0 mg. after 21 days. The wide scatter in initial glucose values probably results from varying degrees of exhaustion. Bacterial attack appears to reduce subsequent glucose disappearance. Ribose increase results from muscle ribosidase action (Tarr, 1955), and subsequent decrease in iced fish from combined leaching and bacterial actions.

REFERENCES

Baar, S. (1954). *Biochem. J.* **58**, 175.
Hodgkiss, W. & Jones, N. R. (1955). *Biochem. J.* **61**, iv.
Hulme, A. C. (1953). *Nature, Lond.*, **171**, 610.
Strange, R. E., Dark, F. A. & Ness, A. G. (1955). *Biochem. J.* **59**, 172.
Tarr, H. L. A. (1954). *Food Tech.* (Champaign), **8**, 15.
Tarr, H. L. A. (1955). *Biochem. J.* **59**, 386.

DEMONSTRATIONS

Analysis of Urinary 17-Oxosteroids by Gradient Elution. By A. E. Kellie. (*Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, W.* 1)

A Modified Electrolytic Desalting Apparatus. By B. J. Stevens, I. Smith and J. B. Jepson. (*Courtauld Institute of Biochemistry, Middlesex Hospital, W.* 1)

The disadvantages of previous designs of electrolytic desalting apparatus (Astrup, Stage & Olsen, 1951; Dent, 1951) have been overcome in a modified version with a high operational stability which will accommodate small volumes (0·5–3 ml.) and maintain the temperature of the sample below 30°. The maximum conversion of arginine to ornithine under these conditions is only 2%.

The semipermeable membrane consists of a viscose bottle seal (VISKAP—Viscose Development Co. Ltd., Chancery Lane, W.C. 2). These are available ready shaped, are easily attached, never leak or split, and conform well to the requirement for a thick membrane for the charge reversal of acidic amino acids (Consden, Gordon & Martin, 1945).

Examples of desirable and undesirable chemical changes induced by electrolytic desalting are shown.

REFERENCES

Astrup, T., Stage, A. & Olsen, E. (1951). *Acta chem. scand.* **5**, 1343.
Consden, R., Gordon, A. H. & Martin, A. J. P. (1945). *Biochem. J.* **39**, xlvi.
Dent, C. E. (1951). In *Recent Advances in Clinical Pathology*, 2nd ed. p. 252. London: Churchill.

A Simple Fluorimeter Adaptable for the Measurement of Fluorescence in Solution or on Paper Chromatograms. By P. J. Ayres, Sylvia A. Simpson and J. F. Tait. (*Courtauld Institute of Biochemistry and the Department of Physics applied to Medicine, Middlesex Hospital Medical School, W.* 1)

The apparatus consists of a mercury lamp as the source of irradiating light and a photomultiplier tube with sensitive galvanometer as the detector of emitted fluorescence. Provision is made for the insertion of primary and secondary filters, which in the case of the latter may be interference filters. The fluorescence of 3 or 0·5 ml. solutions may be measured or the apparatus can readily be adapted for the quantitative measurement of fluorescent spots on paper chromatograms.

The application of the fluorimeter to the microestimation of Δ^4-$\alpha\beta$-unsaturated ketonic steroids, after their chromatography on paper and development by the soda fluorescent method (Bush, 1952), will be demonstrated.

REFERENCE

Bush, I. E. (1952). *Biochem. J.* **50**, 370.

Zone Electrophoresis of Plasma Proteins. By P. N. CAMPBELL and NANCY E. STONE. (*Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, W.* 1)

The method of zone electrophoresis in columns described by Flodin & Porath (1954) and Porath (1954) has been used for the preparation of rat plasma albumin. The supporting medium was cellulose prepared as follows. Absorbent cotton wool (300 g.) was refluxed for 20 hr. with 4875 ml. of ethanol and 375 ml. of acetyl chloride. The product, a white powder, was filtered and washed with ethanol and water and the larger particles removed either by sieving or by flotation.

After removal of globulin from rat plasma with half-saturated $(NH_4)_2SO_4$, approximately 300 mg. of the dialysed freeze-dried albumin fraction was purified on the column. A borate-phosphate buffer (0·0115M $Na_2B_4O_7$, 0·0155M NaH_2PO_4, $I = 0·05$, pH 8·6) was used. This process can be repeated without re-packing the column. The initial current was 28 mA. and the run was continued for 42 hr. The column was then eluted with buffer, the protein composition of the effluent being determined by its ultraviolet absorption at 280 mμ. Preliminary experiments suggest that yields are substantially quantitative.

We are grateful to Dr Porath for valuable advice in the use of his method for the purpose described.

REFERENCES

Flodin, P. & Porath, J. (1954). *Biochim. biophys. Acta*, **13**, 175.
Porath, J. (1954). *Acta chem. scand.* **8**, 1813.

The Preparation of Low-Sodium Milk for Clinical Use. By C. A. LEWIS. (*Courtauld Institute of Biochemistry, Middlesex Hospital, W.* 1)

Modification of the electrolyte content of milk has been effected by passage through ion-exchange resins. Milks low in potassium (Black & Milne, 1952) and low in calcium (Ferguson & McCowan, 1954) have been prepared and put to satisfactory clinical use.

Milk is allowed to flow at 1–2 ml./min. through a 70 g./18 cm. column of Zeokarb 225 (K^+ form); K^+ is exchanged for the Na^+ in the milk, where the concentration falls from 20 to 3 meq./l. At greater flow rates the exchange is less favourable. At the same time Ca^{2+} and Mg^{2+} are retained on the resin.

After the passage of each litre of milk the column is returned to the K^+ form. 20% KCl is not a suitable regenerant as the divalent ions are not displaced and the effective exchange capacity of the column falls progressively. Treatment involving HCl is complicated by the fact that milk is very sensitive to traces of residual acid. Complete regeneration in one step is achieved by a 0·5M solution of the K_4 salt of ethylenediaminetetraacetic acid, which is a powerful complexing agent for polyvalent cations. This simple technique is readily adaptable for use in a diet kitchen.

The treated milk has a bitter taste, reminiscent of KCl, but the palatability can be greatly improved if the treated milk is mixed with a small amount of untreated milk to the limit of allowable Na^+ intake.

REFERENCES

Black, D. A. K. & Milne, M. D. (1952). *Lancet*, i, 244.
Ferguson, A. W. & McGowan, G. K. (1954). *Lancet*, i, 1272.

[*The Editors of the Biochemical Journal accept no responsibility for the Reports of the Proceedings of the Society. Abstracts of papers read are published as received from the authors.*]

PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **346th Meeting** *of the Biochemical Society was held in St Thomas's Hospital Medical School, London, S.E.* 1 *on* 16*th December*, 1955, *at* 2 *p.m., when the following papers were read:*

COMMUNICATIONS

The Metabolism of 2-Methylnaphthalene. By A. J. GRIMES and L. YOUNG. (*Department of Biochemistry, St Thomas's Hospital Medical School, London, S.E.* 1)

Although studies have been made of the metabolism of toluene and of the di- and tri-methylbenzenes, little is known of the chemical changes which methyl-substituted polycyclic hydrocarbons undergo in the animal body. Special interest attaches to the metabolism of these latter compounds because certain of them show carcinogenic activity. The present report is concerned with the metabolism of 2-methylnaphthalene in the rabbit, rat, guinea pig and mouse.

The compound was administered to rabbits and rats by stomach tube and to guinea pigs and mice by intraperitoneal injection. The urine of the dosed animals contained a variety of metabolites of the hydrocarbon. It was found that, as with toluene, 2-methylnaphthalene is oxidized in the animal body to the corresponding carboxylic acid, for examination of the urine of the dosed animals by paper chromatography showed that 2-naphthoic acid was excreted by the four species studied. This was confirmed in the case of rabbits and rats by the isolation of the acid from the urine. Evidence for the excretion of conjugated 2-naphthoic acid was obtained with all four species and 2-naphthuric acid was isolated from the urine of rats.

2-Methylnaphthalene was found to undergo hydroxylation in all four species, for their urine when examined by paper chromatography showed the presence of 7-methyl-1-naphthol and 7-methyl-2-naphthol, a change analogous to the conversion of naphthalene to 1- and 2-naphthol by these same four species (Corner & Young, 1954). Like naphthalene (Young, 1947), 2-methylnaphthalene was also found to give rise to a dihydrodiol and to a metabolite which yields the original hydrocarbon when decomposed by acid. The structure of the dihydrodiol has been investigated and it is concluded that the compound is 1:2-dihydro-7-methylnaphthalene-1:2-diol. This finding is of interest because no methyl-substituted hydrocarbon has hitherto been shown to give rise to a dihydrodiol in the animal body. The dosed animals of all four species excreted this compound.

It has become apparent, therefore, that whereas the metabolism of 2-methylnaphthalene resembles that of toluene with respect to the oxidation of the methyl group, it resembles naphthalene rather than toluene in giving rise to phenolic derivatives and a dihydrodiol.

REFERENCES

Corner, E. D. S. & Young, L. (1954). *Biochem. J.* **58**, 647.
Young, L. (1947). *Biochem. J.* **41**, 417.

The Lipid (Ether- and Ethanol-extractable Material) of the Livers of Cholesterol-fed Rats. By J. B. M. RATTRAY and R. P. COOK. (*Department of Biochemistry, Queen's College, Dundee, University of St Andrews*)

The livers of rats fed a diet containing 16·6% olive oil and 1·6% cholesterol were dried *in vacuo* and extracted with ether and with ethanol. The total extract was separated by treatment with acetone at varying temperatures into soluble and insoluble fractions which were further separated by adsorption chromatography on silica gel into seven primary fractions. These fractions were further separated by formation of urea adducts and separation by various solvents into final fractions.

The general composition of the main final fractions and yields (as percentage total extract) were: hydrocarbons and higher alcohols forming urea adducts, 2; cholesteryl esters of saturated fatty acids, 2; cholesteryl esters of fatty acids, 24—this fraction contains also free cholesterol and cholestanol; free fatty acids forming urea adduct, 1; glycerides of saturated fatty acids, 19, and of unsaturated fatty acids, 20; phospolipins, etc., 20; water-soluble components, 11. Evidence for the nature of these fractions will be given.

Minor fractions containing 'fast-acting' sterols (0·2%) and fractions with absorption maxima indicative of dienes and dienones (cf. Cain & Morton, 1955) were obtained.

This work was aided by an expenses grant from the Scottish Hospitals Research Endowment Trust.

REFERENCE

Cain, J. C. & Morton, R. A. (1955). *Biochem. J.* **60**, 274.

Antimycin A in the Study of Succinic Oxidase in Heart-muscle Preparations Deficient in Inorganic Phosphate. By M. B. THORN. (*Department of Biochemistry, St Thomas's Hospital Medical School, London, S.E.* 1)

Experiments previously reported (Thorn, 1955) suggested that the antimycin-inhibitable factor in Keilin & Hartree heart-muscle preparations is freely available to 'succinic dehydrogenase'. This being a property of some interest in a structurally bound system, an attempt has been made to plot the succinic oxidase activity of a given quantity of heart-muscle preparation against increasing concentrations of antimycin-inhibitable factor. Since antimycin A appears to combine stoicheiometrically with the factor (Chance, 1952; Potter & Reif, 1952) it seemed justifiable to use a decreasing scale of μg. antimycin A present per mg. of heart-muscle preparation as an indirect measure of increasing concentrations of factor. From collected data of many experiments a complex curve was obtained, approaching sigmoid form, consisting of a straight line leading into a hyperbola. The hyperbolic portion can be transformed into a straight line by the methods of Lineweaver & Burk (1934). It is unlikely that a complex curve of this character would result from the presence in heart-muscle preparations of a substance competing with the factor for antimycin A. Rather, the curve suggests that, provided sufficient uninhibited factor is present (about 25%), the factor behaves as a coenzyme relatively freely available as hydrogen (electron) acceptor or donator. The maximum velocity was calculated from the Lineweaver–Burk plot, being about 6% greater than the activity of uninhibited succinic oxidase, and also a quantity corresponding to the Michaelis constant, which although of doubtful absolute significance, may be regarded as an inverse measure of the 'affinity' or 'accessibility' of the factor to an adjacent component of the system.

Histidine (Bonner, 1954) and cytochrome *c*, added either separately or together to preparations in media deficient in inorganic phosphate, simultaneously increased the succinic oxidase activity of a control sample and decreased the percentage inhibition of a partially antimycin-inhibited sample. This observation suggests that the factor lies in that part of the succinic oxidase system in which the 'mutual accessibility' (Keilin & Hartree, 1949) of the components is impaired in the near absence of inorganic phosphate. The results of using antimycin A in determining 'maximum velocities' and 'Michaelis constants', as described above, for preparations in low-phosphate media, will be discussed.

REFERENCES

Bonner, W. D. jun. (1954). *Biochem. J.* **56**, 274.
Chance, B. (1952). *Nature, Lond.*, **169**, 215.
Keilin, D. & Hartree, E. F. (1949). *Biochem. J.* **44**, 205.
Lineweaver, H. & Burk, D. (1934). *J. Amer. chem. Soc.* **56**, 658.
Potter, V. R. & Reif, A. E. (1952). *J. biol. Chem.* **194**, 287.
Thorn, M. B. (1955). *Biochem. J.* **60**, xxxvi.

The Effects of Salicylate on Glycogen Content and Oxygen Uptake of the Isolated Rat Diaphragm. By M. J. H. SMITH and SHIRLEY W. JEFFREY. (*Department of Chemical Pathology, King's College Hospital Medical School, London, S.E.* 5)

The injection of salicylate into normal rats produces a marked depletion of liver and muscle glycogen (Lutwak-Mann, 1942; Winters & Morrill, 1955) and an increased oxygen uptake (Meade, 1954). Salicylate has also been reported to cause a number of *in vitro* effects such as diminished glycogen levels in rat-liver slices (Smith, 1955) and increased oxygen uptake in liver and brain preparations (Sproull, 1954; Fishgold, Field & Hall, 1951). The present work is concerned with a study of the *in vitro* effects of salicylate on the glycogen content and oxygen uptake of the isolated rat diaphragm.

Under aerobic conditions, whether a net synthesis or net breakdown of glycogen occurred in the control diaphragms, 5×10^{-3}M salicylate caused a rapid disappearance of glycogen accompanied by an increased production of lactic acid. These effects were reduced at salicylate concentrations of 1×10^{-3} and 5×10^{-4}M and were absent at a concentration of 1×10^{-4}M.

In the presence of 5×10^{-3}M salicylate, the rate of oxygen uptake always showed a marked increase for the initial 30–60 min. of the experiment and then gradually declined, whereas variable effects were obtained with lower concentrations of salicylate.

Under anaerobic conditions 5×10^{-3}M salicylate caused a decrease in glycogen content and an increased lactic acid production. The anaerobic data indicate that the effect of salicylate on glycogen breakdown is not dependent on an increased oxygen uptake although the two effects may be related.

We wish to thank the Diabetic Association for a grant from the Lund Research Fund.

REFERENCES

Fishgold, J. T., Field, J. & Hall, V. E. (1951). *Amer. J. Physiol.* **164**, 727.
Lutwak-Mann, C. (1942). *Biochem. J.* **36**, 706.
Meade, B. W. (1954). *Ann. rheum. Dis.* **13**, 60.
Smith, M. J. H. (1955). *Biochem. J.* **59**, 52.
Sproull, D. H. (1954). *Brit. J. Pharmacol.* **9**, 262.
Winters, R. W. & Morrill, M. F. (1955). *Proc. Soc. exp. Biol., N.Y.*, **88**, 409.

The Immunological Properties of a Purified Human Fibrinogen. By E. A. Caspary. (*The Lister Institute, London, S.W.* 1)

An antiserum was obtained by immunizing rabbits intravenously with purified human fibrinogen (Kekwick, Mackay, Nance & Record, 1955) showing only one component in the ultracentrifuge and on electrophoresis, but slightly polydisperse by boundary-spreading methods. The clottable nitrogen of the preparation was 97%; contamination with plasminogen or plasmin was negligible.

To eliminate the effect of thrombin when using the antiserum, the animals were bled into trisodium citrate (1/10 vol. 3·8%) and the plasma defibrinated by the addition of a minimal amount of purified thrombin which was subsequently neutralized by the natural antithrombin. Precipitin tests were made in a citrate–saline solution. The titre after two courses of immunization was 1:32 at 37° with 0·1% (w/v) fibrinogen. In tests keeping the antibody constant, free fibrinogen was detected in the antigen excess region of the antigen–antibody reaction with thrombin, and free antibody in the antibody excess region by titrating with fibrinogen. When tests with plasma fractions were made, a strong reaction was obtained with β-globulin, weak with γ-globulin and no visible effect with albumin and α-globulin. Antiserum absorbed with β-globulin gave a narrower precipitin zone and a fourfold drop in titre. Fibrinogen digested with plasmin to complete loss of clottability reacted with the antiserum with a narrower zone of precipitation; antiserum absorbed with this material failed to react with fibrinogen.

Diffusion in agar (Ouchterlony, 1949) with several fibrinogen preparations showed one major component and one or more faint lines at high antigen concentration. It was evident that some of these lines were caused by α-, β- and γ-globulins.

Plasmin-digested fibrinogen diffuses at a greater rate than fibrinogen and immunological identity cannot be established by this method. An experiment with bovine fibrinogen gave a weak cross-reaction with the antiserum.

One major and two minor components were demonstrated by electrophoresis in agar (Grabar & Williams, 1953) and antibodies to many plasma proteins were detected in the antiserum. Anti-globulin activity was proved by the 'Anti Human Globulin Test' (Coombs, Mourant & Race, 1945).

Thrombin acts on fibrinogen in the presence of the antiserum to give a gelatinous precipitate; the antiserum has no effect on plasma prothrombin conversion.

Immunization of rabbits with physicochemically 'pure' human fibrinogen induced an antibody response which indicates trace contamination with several plasma proteins. The product obtained by digesting fibrinogen with plasmin retained the immunological properties of fibrinogen.

REFERENCES

Coombs, R. R. A., Mourant, A. E. & Race, R. R. (1945). *Brit. J. exp. Path.* **26**, 255.
Grabar, P. & Williams, C. A. (1953). *Biochim. biophys. Acta*, **10**, 193.
Kekwick, R. A., Mackay, M. E., Nance, M. H. & Record, B. R. (1955). *Biochem. J.* **60**, 671.
Ouchterlony, O. (1949). *Acta path. microbiol. Scand.* **26**, 507.

The Mechanism of Growth of Starch Granules in the Potato Tuber, Studied with Carbon-14. By N. P. Badenhuizen and R. W. Dutton. (*Postgraduate Medical School, Ducane Road, London, W.* 12)

The classical work of A. Meyer (1895) indicated that the growth of starch granules takes place by apposition. The theory implies structural equality of the layers, as has in fact been found (Badenhuizen, 1955). The process could be one of rapid rhythmic crystallization, or one of slow deposition of starch. The alternative theory of intussusception requires the assumption of an outer membrane with special properties. The problem can only be solved by following growth directly. The stripping film technique of autoradiography opens up this possibility.

Using an apparatus developed by one of us (Dutton, 1955) potato plants were allowed to photosynthesize in an atmosphere containing $^{14}CO_2$. Initial CO_2 concentrations from 0·1 to 1·5% were used and activities from 75 to 400 μc incorporated. Activity measurements on various parts of the plant surface indicated a rapid decrease of activity in the leaves and a downward transport to the tubers of active material, which was shown by paper chromatography of extracts to be mainly sucrose. Slices were cut from the tubers and examined by autoradiography. Even after 8 days tubers on the same plant showed a wide range of activities, some being highly active, whilst some were inactive, and others intermediate. Starch was then isolated from the tubers and the granules were examined by the stripping film technique of Pelc (1947).

Native granules showed greater activity at the distal end and at the periphery. After breaking the granules, or gelatinizing them in the presence of saliva, the exposed inner mass was found to be inactive. Corrosion of the granule surface by saliva removed activity, and this process started at the proximal end. Activity was therefore located in a newly deposited layer coating the granule. It was further found that the cytoplasm became active first and then, very gradually, the starch granules. There were also indications that in an intermediate stage amyloplasts became highly active.

We conclude that growth takes place by apposition, and that it may be a very slow process which is entirely dependent on the supply of carbohydrate.

We thank the Royal Society and the Nuffield Foundation (N. P. B.) and the Medical Research Council (R. W. D.) for grants.

REFERENCES

Badenhuizen, N. P. (1955). *Protoplasma.* (In the Press.)
Dutton, R. W. (1955). Ph.D. Thesis, London University.
Meyer, A. (1895). *Untersuchungen über die Stärkekörner.* Jena: Fischer.
Pelc, S. R. (1947). *Nature, Lond.*, **160**, 749.

Anti-metabolites from Vitamin B_{12}. By E. Lester Smith, L. F. J. Parker and Dorothy E. Gant. (*Glaxo Laboratories Limited, Greenford, Middlesex*)

The mono-, di- and tri-carboxylic acids resulting from mild acid hydrolysis of vitamin B_{12} can all be reconverted into the vitamin by reaction in anhydrous dimethylformamide solution first with ethyl chloroformate and triethylamine and then with ammonia (Armitage *et al.* 1953). It was to be expected that treatment of the mixed anhydrides with an amine instead of ammonia would yield the corresponding amides, and that some of these might have anti-vitamin activity.

The reaction mixtures were purified by electrophoresis on paper, to remove unreacted acids and some side-reaction products. The red neutral zones were further purified by repeated paper chromatography with wet *sec.*-butanol containing traces of acetic and hydrocyanic acids. When amino acids or diamines were used, the products were acidic or basic, respectively, and appeared in the appropriate electrophoresis zones.

Crystalline products resulted from the monobasic acids (mixture of isomers or, in a few instances, single isomers) and the 8 amino compounds marked with an asterisk in the list below; other amides did not crystallize. Rigorous purification of the acids used, and of the products, was necessary to remove traces of vitamin B_{12} before anti-vitamin activity could be demonstrated by plate assays with the B_{12}-requiring *Escherichia coli* mutant (see next abstract).

Anti-B_{12} activity was shown by mono-amides of the following: methylamine*, ethylamine*, monoethanolamine, ethylenediamine, dimethylamine, diethylamine*, piperidine*, phenylethylamine, cyclohexylamine, aniline* and sulphanilic acid. Of these, the methylamide appeared the most active, with an inhibition index of about 150. Some larger amines, amylamine*, octylamine and β-naphthylamine gave inactive compounds as did the amino-acids glycine* and alanine and *p*-aminobenzoic acid*. The di-anilides of the dibasic acids were inactive: the tribasic acid gave a weakly active triethylamide and an inactive and almost insoluble tri-anilide. The ethylamides of the monobasic acids without nucleotide were active.

REFERENCE

Armitage, J. B., Cannon, J. R., Johnson, A. W., Parker, L. F. J., Smith, E. Lester, Stafford, W. H. & Todd, A. R. (1953). *J. chem. Soc.* p. 3849.

The Anti-vitamin B_{12} Activity of Some Compounds related to Cobalamin. By W. F. J. CUTHBERTSON, J. GREGORY, P. O'SULLIVAN and H. F. PEGLER. (*Glaxo Laboratories Limited, Greenford, Middlesex*)

Methods have been devised for detecting and assessing compounds that may antagonize the utilization of cyanocobalamin by vitamin B_{12}-dependent organisms.

Qualitative tests were carried out in agar media with *Escherichia coli* (Harrison, Lees & Wood, 1951) and sometimes with *Lactobacillus leichmannii* (Skeggs *et al.* 1950) as well. Into cups cut about 8 mm. apart in vitamin B_{12} assay plates, seeded with the appropriate organism, 3 drops of solution (0·1 μg./ml.) were placed—cyanocobalamin in one cup and test solution (1–1000 μg./ml.) in the other. After overnight incubation the zones of growth were noted. Inert substances did not modify the circular zones of growth around the cups containing the vitamin. Non-specific inhibitors, e.g. phenol, produced clearly defined circular zones of inhibition, cutting sharp circular arcs from the growth zones produced by the vitamin in adjacent cups. Competitive antagonists were expected to depress growth most at low and least at high vitamin concentrations. The inhibition due to such substances should therefore rapidly decrease towards the centre of the growth zones round the adjacent cups and extend further round the edges than when non-specific growth inhibitors are tested. Such effects were in fact found.

The cup-plate method is only qualitative; quantitative assessment of the inhibition index (Shive, 1950) was carried out in fluid media (Harrison *et al.* 1951; Burkholder, 1951; Skeggs *et al.* 1950) by use of graded levels of both vitamin B_{12} and the antagonist. Of the compounds tested the methylamide of the mixed monobasic acids derived from cobalamin (Smith, Parker & Gant, 1955) was the most active (inhibition index of 150 with *E. coli*); the anilide of acid E2 and the diethylamide of the mixed monobasic acids were less active (inhibition indices 2000–3000 with *E. coli*).

Vitamin B_{12}-deficient rats were given varied levels of vitamin and antagonist. In the individual tests the antagonists depressed growth, though not significantly, but when all results on the different antagonists were combined a significant growth depression ($0{\cdot}05 > P > 0{\cdot}001$) could be demonstrated at doses of 0·1–3 μg./day. The data are limited owing to the small amounts of material available and are not sufficient to establish whether or not these effects can be reversed by cyanocobalamin. Attempts were made to detect substances inhibiting vitamin B_{12} synthesis but no positive results were obtained.

REFERENCES

Burkholder, P. (1951). *Science*, **114**, 459.
Harrison, E., Lees, K. A. & Wood, F. (1951). *Analyst*, **76**, 696.
Shive, W. (1950). *Ann. N.Y. Acad. Sci.* **52**, 1212.
Skeggs, H. R., Nepple, H. M., Valentik, K. A., Huff, J. W. & Wright, L. D. (1950). *J. biol. Chem.* **184**, 211.
Smith, E. L., Parker, L. F. J. & Gant, D. (1955). Preceding communication.

The Estimation of Oestrone, Oestradiol-17β, and Oestriol in Human Urine by Gradient Elution Partition Chromatography. By ELSIE H. AITKEN and J. R. K. PREEDY (introduced by C. J. O. R. MORRIS). (*Medical Unit, London Hospital, E.* 1)

Twenty-four hour urine aliquots (about 3% of total volume) were extracted by the procedure of Engel *et al.* (1950), except that autoclaving was replaced by refluxing for 0·5 hr. in 1·8N-H_2SO_4. The dried extract was transferred quantitatively to a partition column, 15 cm. long, 0·57 cm. in internal diameter, packed with Hyflo Super-Cel as supporting phase. The Super-Cel had previously been mixed with stationary phase (70 vol. CH_3OH—30 vol. distilled water) in the proportion 0·66 ml./g. Super-Cel. Chromatography was begun with a mobile phase containing 32% (v/v) CCl_4—68% petroleum ether (boiling range 60–80°). Fifteen 1 ml. fractions were collected. The mobile phase was then changed to 23% (v/v) CCl_4—12% $CHCl_3$—65% petroleum ether. A further fifteen 1 ml. fractions were collected. The mobile phase was then again changed to 45% $CHCl_3$—55% petroleum ether. A further twenty-four 1 ml. fractions were collected. The dried fractions were estimated for oestrogen content by fluorimetry (Engel *et al.* 1950). Automatic changes of mobile phase were accomplished by attaching to the column a U-shaped glass reservoir, the two limbs of which, containing 32% CCl_4—68% petroleum ether and 45% $CHCl_3$—55% petroleum ether respectively, were constructed of tubing of the necessary lengths and internal diameters to obtain successively the volumes and compositions of mobile phase required. This chromatographic procedure enabled the three oestrogens to be separated not only from one another, but also from

the large amounts of fluorescent interfering material present in the urine extracts, by means of a single partition column allowed to run overnight. Preliminary recoveries of pure crystalline oestrone, oestradiol-17β and oestriol, 0·2–1·0 μg. of each added to male and female urine aliquots, were as follows (means and ranges): oestrone 86% (81–91), oestradiol-17β 88% (83–94), oestriol 79% (70–85). Normal urine oestrogen outputs so far obtained were (μg./day): male, oestrone 2·4–4·0, oestradiol-17β 2·0, oestriol $<2·0$; female, oestrone 2·9–5·1, oestradiol-17β $<2·0$; oestriol 2·9–7·3.

REFERENCE

Engel, L. L., Slaunwhite, W. R., Carter, P. & Nathanson, I. T. (1950). *J. biol. Chem.* **185**, 255.

Inhibition of Dihydrocozymase Oxidase Activity of Pig-Heart Muscle and Bacterial Preparations by 2-Heptyl-4-hydroxyquinoline-*N*-oxide. By F. L. Jackson* (*King's College Hospital Medical School, London*) and J. W. Lightbown (introduced by J. H. Humphrey) (*National Institute for Medical Research, Mill Hill, London*)

The Keilin and Hartree type of heart muscle succinic oxidase preparation will also oxidize dihydrocozymase (DPNH), and this process can be followed by measuring the decrease in absorption at 340 mμ., as described by Slater (1950). Using this method, we have been able to demonstrate inhibition of DPNH oxidation when 2-heptyl-4-hydroxyquinoline-*N*-oxide (heptyl-*N*-oxide) was added to the preparation. Concentrations of 1·5 μg. of heptyl-*N*-oxide/ml. produced 90% inhibition of activity. Similar experiments with cell-free preparations of *Staphylococcus aureus* showed approximately the same degree of inhibition.

With the heart-muscle preparation, the anaerobic Thunberg technique with methylene blue was used to investigate possible effects on diaphorase activity. Concentrations of heptyl-*N*-oxide of 5 μg./ml. did not increase the methylene-blue reduction time.

In heart muscle succinic oxidase system, the site of action appears to be between succinic dehydrogenase and cytochrome *c* (Lightbown & Jackson, 1954). Slater (1950), who excludes cytochrome *b* from the DPNH oxidase system, has postulated that diaphorase is linked to cytochrome *c* through an intermediate factor, the same factor linking cytochromes *b* and *c* in the succinic oxidase system. If this is so, the factor, which has been suggested by Potter & Reif (1952, 1954) as the site of action of antimycin A, might also be the site of action of the heptyl-*N*-oxide. However, we have observed spectroscopically that cytochrome *b* is rapidly and strongly reduced by DPNH in the presence of either heptyl-*N*-oxide or antimycin A, and that cytochromes *c* and *a* are only slowly reduced under these conditions.

Since the inhibitors interfere with electron transport between cytochromes *b* and *c*, strong reduction of cytochrome *b* by DPNH would suggest that cytochrome *b* is on the pathway for DPNH oxidation. While their site of action might be on a factor between cytochromes *b* and *c*, it is possible that the inhibitors might react with cytochrome *b* itself, so as to prevent its oxidation by way of the cytochrome *c*–cytochrome oxidase pathway, but not its reduction by succinic dehydrogenase or diaphorase.

* Cowburn Research Fellow.

REFERENCES

Lightbown, J. W. & Jackson, F. L. (1954). *Biochem. J.* **58**, xlix.
Potter, van R. & Reif, A. E. (1952). *J. biol. Chem.* **194**, 287.
Potter, van R. & Reif, A. E. (1954). *Arch. Biochem. Biophys.* **48**, 1.
Slater, E. C. (1950). *Biochem. J.* **46**, 499.

Analysis of Steroids in Adrenal Venous Blood and in Urine in a Case of Virilism. By I. E. Bush (*St Mary's Hospital, London, W.* 2), J. Swale and J. Patterson (*Charing Cross Hospital, London, W.C.* 2)

The work of Callow first demonstrated the importance of dehydro*iso*androsterone (DHIA) as a human metabolite (Callow & Callow, 1939). This substance is the preponderant 17-ketosteroid in most normal people (Lieberman & Teich, 1953). There is strong evidence that DHIA is derived from the adrenal cortex, but Lieberman & Teich (1953) suggest that it is not secreted by the adrenal as such, but results from a precursor. We have obtained evidence that this suggestion is largely correct.

Adrenal venous blood was obtained at operation for adrenalectomy on a case of post-pubertal virilism; 24 hr. urine collections were made on this

day and during her stay in hospital. ACTH (gel 2 × 50 U.) was administered during the day of operation to ensure maximal secretion rates. 30 ml. blood was collected in 15 min. from the left adrenal vein and analysed by paper chromatography (Bush, 1952, 1954) after extraction in the usual way. The sample contained approximately (±20%) 67 μg. hydrocortisone (F), 67 μg. corticosterone, 28 μg. 11-β-hydroxyandrost-4-ene-3:17-dione, 28 μg. DHIA, 12 μg. androsterone. Urinary excretion (mg./24 hr.) for that day was: tetrahydrocortisone, 2·90; tetrahydroF, 1·2; androsterone, 4·35; etiocholanolone, 2·70; DHIA, 5·6; 11-β-hydroxyetiocholanolone, 0·17; 11-ketoetiocholanolone, 0·45; 11-β-hydroxyandrosterone, 0·74.

No peripheral blood was obtained, but it is unlikely that more than 5% of each compound in the blood sample arose from arterial blood (Bush & Sandberg, 1953; Migeon & Plager, 1954).

Back-calculation from the urinary figures using percentage conversion values similar to Dorfman's (1954) for 11-oxygenated 17-ketosteroids and reducing steroids gives a calculated hydrocortisone/11-β-hydroxyandrost-4-ene-3:17-dione secretory ratio of 2·2–3·35 (observed 2·4). The observed hydrocortisone/DHIA ratio (2·4) however would require an excretion of 82–88% unchanged DHIA to account for urinary DHIA. Even allowing for earlier methods, this is an unreasonably high figure (Mason & Kepler, 1946). If a 5% excretion is assumed for DHIA, 4·25% of urinary DHIA would be accounted for by secreted DHIA. This suggests strongly that >90% of urinary DHIA was derived from an unidentified adrenal precursor in our case.

Dorfman's (1954) concepts of the origin of 11-oxygenated 17-ketosteroids receive considerable support from this case in view of the agreement between calculated and observed hydrocortisone/11-β-hydroxyandrost-4-ene-3:17-dione ratios.

We are indebted to Mr L. R. Broster for obtaining this crucial blood sample.

REFERENCES

Bush, I. E. (1952). *Biochem. J.* **50**, 370.
Bush, I. E. (1954). *Recent Progr. Hormone Res.* **9**, 321.
Bush, I. E. & Sandberg, A. A. (1953). *J. biol. Chem.* **205**, 783.
Callow, N. H. & Callow, R. K. (1939). *Biochem. J.* **33**, 931.
Dorfman, R. I. (1954). *Recent Progr. Hormone Res.* **9**, 5.
Lieberman, S. & Teich, S. (1953). *Pharmacol. Rev.* **5**, 285.
Mason, H. L. & Kepler, E. J. (1946). *J. biol. Chem.* **167**, 73.
Migeon, C. & Plager, J. E. (1954). *Recent Progr. Hormone Res.* **9**, 235.

Nucleotide Sequences in Yeast Ribonucleic Acids. By W. E. Cohn and R. Markham. (*University Chemical Laboratory, and Agricultural Research Council Virus Research Unit, Molteno Institute, Cambridge*)

Information as to the variety of sequences of nucleotides existing in ribonucleic acids is dependent upon the isolation and characterization of polynucleotides obtained from them. Exhaustive degradation by pancreatic ribonuclease yields mixtures of polynucleotides containing all those sequences which are resistant to the enzyme. Of the sixteen possible dinucleotide combinations, linked 3′ to 5′ and possessing a free 3′-phosphate, four are found as dinucleotides in ribonuclease digests (AC, AU, GC, GU; A, G, C and U represent adenylic, guanylic, cytidylic and uridylic acids respectively and the 3′–5′ internucleotide link runs from left to right), and four others exist as portions of trinucleotides and larger polynucleotides (AA, AG, GA, GG) (Markham & Smith, 1952; Volkin & Cohn, 1953). The remaining eight are not found in exhaustive ribonuclease digests, due to the specificity of the enzyme, and its transferase activity reduces the significance attached to the finding of such sequences in partial digests. Brief acid hydrolysis, however, has indicated that yeast ribonucleic acids contain the sequences CC, CA and UC and/or CU (Merrifield & Woolley, 1952). This type of hydrolysis was employed by us to find the missing sequences and to determine the relative amounts of isomeric pairs (e.g. GC and CG).

Commercial yeast ribonucleic acid, reprecipitated, was treated for 2·5 min. with 6N-HCl at 20°, neutralized, and separated into known polynucleotide fractions by paper chromatography. From these fractions, pairs of isomeric dinucleotides (e.g. GC and CG) having identical electrophoretic mobilities were isolated by paper electrophoresis, eluted, treated with ribonuclease and rechromatographed. The mononucleotides liberated were identified by chromatography and by spectrum. The unhydrolysed dinucleotide was then hydrolysed and chromatographed to determine its structure.

The results indicate that those sequences not previously found are present in yeast ribonucleic acids in amounts not grossly differing from those of

the other sequences. Thus it would appear that all sequences of dinucleotides are to be found in yeast ribonucleic acids.

One of us (W. E. C.) gratefully acknowledges the support of John Simon Guggenheim Memorial Foundation and Fulbright Fellowships.

REFERENCES

Markham, R. & Smith, J. D. (1952). *Biochem. J.* **52**, 552, 558, 565.
Merrifield, R. B. & Woolley, D. W. (1952). *J. biol. Chem.* **197**, 521.
Volkin, E. & Cohn, W. E. (1953). *J. biol. Chem.* **205**, 767.

Distribution of Pressor Amines and Adenosinetriphosphate (ATP) in Adrenal Medullary Granules. By H. Blaschko, G. V. R. Born, A. D'Iorio and N. R. Eade. (*Department of Pharmacology and Nuffield Institute for Medical Research, University of Oxford*)

Homogenates of the bovine adrenal medulla are known to contain the bulk of their adrenaline and noradrenaline in granules with sedimentation properties similar to those of mitochondria; in these granules the amines are mainly present in a condition in which they are not able immediately to exert their biological action. The 'mitochondrial' fraction can be subdivided into a 'bottom' layer of granules with a relatively high amine content and a 'top' layer less rich in amines (Blaschko, Hagen & Welch, 1955).

Högberg, Hillarp & Nilsson (1955) have recently reported the occurrence of large amounts of ATP in adrenal medullary granules. We have therefore studied the distribution of ATP in fractions obtained from the bovine adrenal medulla, in relation to their content of catechol amines. ATP was determined by the firefly luminiscence test (Born & Bülbring, 1955). It was found that the bottom layer was extremely rich in high-energy phosphate; in three experiments the concentrations of ATP, on the basis of dry weight, were 7·7, 7·9 and 4·5% respectively; in the same experiments the top layer, which is less rich in catechol amines, contained 0·9, 1·2 and 2·0% of ATP.

Upon transfer into hypotonic media the granules are known rapidly to release the catechol amines; this treatment was also found to release the ATP. Resuspension of the bottom fraction in 0·2M sucrose released about one-half of both ATP and catechol amines; in 0·1M sucrose practically all the ATP and catechol amines were found in the supernatant. No evidence for destruction of ATP was obtained in these experiments.

REFERENCES

Blaschko, H., Hagen, P. & Welch, A. D. (1955). *J. Physiol.* **129**, 27.
Born, G. V. R. & Bülbring, E. (1955). *J. Physiol.* **127**, 626.
Högberg, B., Hillarp, N-A. & Nilsson, B. (1955). *3ème Congrès International de Biochimie; Résumé des Communications*, p. 72.

Studies on the Lipolytic System Induced in Plasma by Heparin Injection. By D. S. Robinson. (*Sir William Dunn School of Pathology, University of Oxford*)

Hahn (1943) observed that the intravenous injection of heparin abolished the alimentary lipaemia which is normally present during fat absorption. Subsequent studies on this 'clearing system', induced in plasma by heparin injection, have shown that it represents a lipolytic process in which the triglyceride of the lipid particles is hydrolysed to free fatty acid with the formation of a soluble fatty acid-albumin complex in the plasma (Robinson & French, 1953). The addition of heparin to plasma *in vitro* does not produce an active 'clearing system'.

In the experiments to be reported active plasma, obtained from rats injected with heparin, has been passed through a column of 'De-Acidite F' (Permutit Co. Ltd.), a strongly basic anion exchange resin, at pH 7·4 and 0°. Such treatment results in the removal of heparin from the plasma and the heparin can be recovered by appropriate treatment of the column.

The 'clearing activity' of the effluent from the column has been studied using the techniques already developed in this laboratory. It appears that while the removal of heparin from the plasma does not destroy the enzyme system it does markedly reduce its stability. Thus, after only 10 min. incubation of the effluent at pH 7·4 and 37°, the clearing activity falls to 10% of its initial value. If small quantities of free heparin are added to the effluent prior to its incubation the

enzyme system regains the stability of the original plasma and no detectable fall in activity occurs during incubation for 10 min. at 37°.

This work was carried out during the tenure of the Alan Johnston, Lawrence and Moseley Research Fellowship of the Royal Society.

REFERENCES

Hahn, P. F. (1943). *Science*, **98**, 19.
Robinson, D. S. & French, J. E. (1953). *Quart. J. exp. Physiol.* **38**, 233.

The Effect of Hexoestrol on Cholesterol Metabolism in the Rat. By G. S. Boyd and W. B. McGuire. (*Department of Biochemistry, University of Edinburgh*)

Studies have been made of the effect of the synthetic oestrogen hexoestrol on cholesterol metabolism in the young male rat. Since hexoestrol causes lipid depletion of the adrenal cortex (Vogt, 1945) and apparently induces adrenal insufficiency (Vogt, 1955), metabolic effects of this substance were studied in both intact and adrenalectomized 5–7 week old rats. Rats were fed *ad lib.* during the experiments. Cholesterol was estimated by a micro-modification of the Sperry & Webb method (1950) and total liver glycogen by the anthrone method of van der Vies (1954).

Intact rats receiving 70 μg. hexoestrol/100 g. body weight/day for 6 days progressively lost weight and a marked serum hypocholesterolaemia developed from a control level of 54, to 23 mg. cholesterol/100 ml. serum. Adrenal cholesterol decreased from a control level of 1·26 to 0·820 g./100 g. tissue. Liver slices from control animals and from hexoestrol-treated animals were incubated for 3 hr. at 37° under 100% oxygen in Krebs phosphate solution (Krebs & Henseleit, 1932) in the presence of 3 μM sodium [1-^{14}C]-acetate and the percentage incorporation of acetate into cholesterol determined. It was found that hepatic cholesterol biosynthesis was depressed to 51·7% of the control value. Administration of 70 μg. hexoestrol/100 g. body weight/day for 13 days intensified the effects mentioned above, reducing the serum cholesterol from 73 to 23 mg. cholesterol/100 ml. serum, the adrenal cholesterol from 2·87 to 0·515 g./100 g. tissue and hepatic cholesterol biosynthesis to 11·1% of the control value; liver cholesterol fell from 234 to 161 mg./100 g. tissue and liver glycogen from 9·21 to 5·61 g./100 g. tissue. Pituitaries and adrenals were hypertrophied and the haematocrit value remained at the control level throughout the experiment.

The administration of 50 μg. hexoestrol/100 g. body weight/day for 7 days to adrenalectomized rats resulted in a very marked serum hypocholesterolaemia from 42 mg. cholesterol/100 ml. serum in adrenalectomized control rats to 6 mg. cholesterol/100 ml. serum in hexoestrol treated adrenalectomized rats. Thus hexoestrol appears to inhibit cholesterol synthesis in the male rat and this inhibition may be independent of the adrenal gland.

We thank the Scottish Hospital Endowment Research Fund for a grant.

REFERENCES

Krebs, H. A. & Henseleit, K. (1932). *Hoppe-Seyl. Z.* **210**, 33.
Sperry, W. M. & Webb, M. (1950). *J. biol. Chem.* **187**, 97.
van der Vies, J. (1954). *Biochem. J.* **57**, 410.
Vogt, M. (1945). *J. Physiol.* **104**, 60.
Vogt, M. (1955). *J. Physiol.* **128**, 7P.

[*The Editors of the Biochemical Journal accept no responsibility for the Reports of the Proceedings of the Society. Abstracts of papers read are published as received from the authors.*]

PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **347th Meeting** *of the Biochemical Society was held in the Postgraduate Medical School, Ducane Road, London, W. 12, on 21 January 1956, at 2 p.m., when the following papers were read:*

COMMUNICATIONS

Isolation of Oestradiol-17α and Equol from Pregnant Goats' Urine.

By W. Klyne and A. A. Wright. (*Postgraduate Medical School, London, W. 12*)

The isolation of oestrone (~0·4 mg./l.) and 5β-pregnane-3α:20α-diol (~2 mg./l.) from the urine of pregnant goats has previously been reported (Wright & Klyne, 1955); several 17-oxosteroids were also found.

The study of the non-ketonic phenolic fraction has now yielded oestradiol-17α and equol (*iso*flavan-7:4′-diol; Marrian & Haslewood, 1932; Marrian & Beall, 1935; Anderson & Marrian, 1939). The non-ketonic phenols were submitted to partition between water and benzene-light petroleum (Brown, 1955). The aqueous fraction yielded equol (~10 mg./l.) identified as free phenol, dimethyl ether, diacetate and dibenzoate by m.p., mixed m.p. and infrared spectra. The benzene-light petroleum fraction was chromatographed on alumina; those fractions which gave a positive Kober reaction (Bauld, 1954) were methylated and the methyl ethers again chromatographed. Oestradiol-17α 3-methyl ether was thus readily separated from equol dimethyl ether. The oestradiol-17α 3-methyl ether (~0·1 mg./l.) was identified by m.p., mixed m.p. and infrared spectrum. It gave the characteristic colour reaction with sulphuric acid and acetic acid which distinguishes oestradiol-17α from the 17β-epimer (Kägi & Miescher, 1939).

Equol is probably of dietary origin; *iso*flavones occur, chiefly as glycosides, in a variety of plants including clovers, vetch and soya bean (Warburton, 1954). Genistein (5:7:4′-trihydroxy*iso*flavone) and two of its methyl ethers are weakly oestrogenic (Bradbury & White, 1954).

We thank the Agricultural Research Council for grants and Dr S. J. Folley, F.R.S., and Dr A. T. Cowie (National Institute for Research in Dairying) for gifts of pregnant goats' urine.

REFERENCES

Anderson, E. L. & Marrian, G. F. (1939). *J. biol. Chem.* **127**, 649.
Bauld, W. S. (1954). *Biochem. J.* **56**, 426.
Bradbury, R. B. & White, D. E. (1954). *Vitam. & Horm.* **12**, 207.
Brown, J. B. (1955). *Biochem. J.* **60**, 185.
Kägi, H. & Miescher, K. (1939). *Helv. chim. Acta*, **22**, 683.
Marrian, G. F. & Beall, D. (1935). *Biochem. J.* **29**, 1586.
Marrian, G. F. & Haslewood, G. A. D. (1932). *Biochem. J.* **26**, 1227.
Warburton, W. K. (1954). *Quart. Rev. chem. Soc., Lond.*, **8**, 67.
Wright, A. A. & Klyne, W. (1955). *Abstr. 3rd Int. Congr. Biochem.* p. 139.

The Identification of 5α-Androstane-3β:16-diols as Constituents of Pregnant Mares' Urine.

By R. V. Brooks* and W. Klyne. (*Postgraduate Medical School, London, W. 12*)

Heard & McKay (1939) and Oppenauer (1941) isolated from pregnant mares' urine a hydroxyketone, subsequently shown by Huffman & Lott (1951) to be 3β-hydroxy-5α-androstan-16-one. Brooks, Klyne, Miller & Paterson (1952) isolated from the same source a diol, Compound *MM*, of unknown structure.

Fajkoš & Šorm (1955) and Huffman & Lott (1955) have recently prepared the two 5α-androstane-3β:16α-(and 16β)-diols, and a comparison of the properties described for the 3β:16β-diol with those of Compound *MM* suggested that the two substances might be identical. Thanks to the kindness of Prof. F. Šorm (Czechoslovak Academy of Science, Prague) and Professor M. N. Huffman (Oklahoma Medical Research Foundation), we have been able to compare the two compounds directly. The melting points, mixed melting

* Present address: St Thomas's Hospital Medical School, London, S.E. 1.

points and infrared spectra of the diols, diacetates and dibenzoates show that Compound *MM* is indeed 5α-androstane-3β:16β-diol. Compound *MN* is the 3β:16α-isomer.

3:16-Dihydroxysteroids of the androstane series have not previously been reported in nature, although 5α-pregnane-3β:16α:20β-triol (Haslewood, Marrian & Smith, 1934; Hirschmann, Hirschmann & Daus, 1949) is one of the major constituents of the triol fraction from mares' urine and 3:16:17-triols (including oestriol) are well-known constituents of human urine (for references see Hirschmann, 1955; Marrian & Bauld, 1955).

We thank the Agricultural Research Council for grants and we are indebted to Messrs Ayerst, McKenna and Harrison (Montreal), British Drug Houses Ltd. (London), Ciba (Basel) and Organon (Oss, Netherlands) for generous gifts of concentrates of pregnant mares' urine.

REFERENCES

Brooks, R. V., Klyne, W., Miller, E. & Paterson, J. Y. F. (1952). *Biochem. J.* **51**, 694.
Fajkoš, J. & Šorm, F. (1955). *Chem. Listy*, **49**, 723.
Haslewood, G. A. D., Marrian, G. F. & Smith, E. R. (1934). *Biochem. J.* **28**, 1316.
Heard, R. D. H. & McKay, A. F. (1939). *J. biol. Chem.* **131**, 371.
Hirschmann, H. (1955). In *The Hormones*, ed. by Pincus, G. & Thimann, K. V., vol. 3, p. 522. New York: Academic Press.
Hirschmann, H., Hirschmann, F. B. & Daus, M. A. (1949). *J. biol. Chem.* **178**, 751.
Huffman, M. N. & Lott, M. H. (1951). *J. Amer. chem. Soc.* **73**, 878.
Huffman, M. N. & Lott, M. H. (1955). *J. biol. Chem.* **215**, 633.
Marrian, G. F. & Bauld, W. S. (1955). *Biochem. J.* **59**, 136.
Oppenauer, R. (1941). *Hoppe-Seyl. Z.* **270**, 97.

The Synthesis of Citrulline in Liver Particles and Extracts. By M. Shilo and D. E. Hughes. (*Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry, University of Oxford*)

Extracts prepared from an acetone powder of the particulate fraction of rat-liver homogenates synthesize citrulline from ornithine, CO_2, ammonia and ATP if carbamoylglutamate or acetylglutamate is added (Grisolia & Cohen, 1952). More active extracts are easily obtained by crushing the particles in a bacterial press (Hughes, 1951) at −15° and removing the coarse debris by centrifugation at 18 000 *g*. Extracts of crushed particles, like those from acetone powders, do not synthesize citrulline when acetyl- or carbamoyl-glutamate is replaced by glutamate. In the presence of acetylglutamate the activity of crushed material is up to five-fold greater than that of the intact particles. The activity of the extracts is further increased when the ATP concentration is raised from 0·004M to 0·012M; such an increase does not increase synthesis in the particles. Ageing the particles at 0° for 60 min. or treatment with a non-ionic detergent (Gemex G 0·2%) also increases the rate of synthesis. In this case, however, the enzymes still remain in the particles. These results suggest that one of the components of the system, probably ATP (cf. Bartley & Davies, 1954), is not freely accessible to the enzymes in the particles. Both particles and extracts synthesize citrulline from ornithine and carbamoyl phosphate in the absence of ATP, NH_3, CO_2 and the glutamate derivative (cf. Jones, Spector & Lipmann, 1955). In the particles the rate of synthesis from carbamoyl phosphate is about ten times as fast as from ammonia and CO_2 under optimal conditions, but in the extracts is only slightly greater than with NH_3 and CO_2.

It has previously been suggested (Feldott & Lardy, 1951) that the synthesis of citrulline is decreased by biotin deficiency in rat-liver particles because the vitamin is concerned in the activation of glutamate. We have found that particles from biotin-deficient liver are more readily changed by ageing at 0° than are the particles from normal livers. Ageing decreases the rate of synthesis with glutamate and increases it with acetylglutamate. It thus reproduces the effect previously ascribed to biotin deficiency and makes it difficult to decide whether biotin is concerned directly in the synthesis of citrulline or whether the decrease in synthesis with glutamate and its reversal with acetylglutamate is due to a more indirect effect. It is of further interest in this connexion that biotin has no effect on the synthesis of citrulline by extracts of *Streptococcus faecalis*.

REFERENCES

Bartley, W. & Davies, R. E. (1954). *Biochem. J.* **57**, 37.
Feldott, G. & Lardy, H. A. (1951). *J. biol. Chem.* **192**, 447.
Grisolia, S. & Cohen, P. P. (1952). *J. biol. Chem.* **198**, 561.
Hughes, D. E. (1951). *Brit. J. exp. Path.* **32**, 97.
Jones, M. E., Spector, L. & Lipmann, F. (1955). *J. Am. chem. Soc.* **77**, 819.

The Conversion of 5-Bromosalicylohydroxamic acid to 5-Bromosalicylamide *in vivo*. By W. M. McIsaac and R. T. Williams. (*Department of Biochemistry, St Mary's Hospital Medical School, London, W.* 2)

5-Bromosalicylohydroxamic acid ('T40') has been shown to be tuberculostatic (Urbanski, Hornung, Slopek & Venulet, 1952; Urbanski, 1953). When fed to rabbits we have found that this compound (which is non-fluorescent) gives rise to two main metabolites in the urine, both of which have been identified as glucosiduronic acids. The one found in largest amount has been identified as 4-*bromo*-2-*carbamoylphenyl glucosiduronic acid* (m.p. 213–214°; $[\alpha]_D$ −75° in 0·1N-NaOH; Found: C, 39·6; H, 3·7; N, 3·7; Br, 20·2. $C_{13}H_{14}O_8NBr$ requires C, 39·8; H, 3·6; N, 3·6; Br, 20·4%). On hydrolysis with β-glucuronidase it yielded 5-bromosalicylamide (which is fluorescent) and was identical with the glucuronide (m.p. and mixed m.p. 213–214°) prepared by feeding rabbits with 5-bromosalicylamide. The second glucuronide was identified as 4-*bromo*-2-*hydroxycarbamoylphenyl glucosiduronic acid* (m.p. 192° and $[\alpha]_D$ −71° in 0·1N-NaOH; Found: C, 36·2; H, 3·6; N, 3·2; H_2O, 4·2. $C_{13}H_{14}ONBr,H_2O$ requires C, 36·6; H, 3·8; N, 3·3; H_2O, 4·2%). On hydrolysis with β-glucuronidase, this compound yielded 5-bromosalicylohydroxamic acid, whilst on acid hydrolysis it gave 5-bromosalicylic acid and the hydroxamic acid according to the strength of acid used and the time of hydrolysis.

The unsubstituted salicylohydroxamic acid (non-fluorescent) is also tuberculostatic; however, it is not converted to salicylamide (fluorescent) in the rabbit. Its main metabolite is 2-*hydroxycarbamoylphenyl glucosiduronic acid* (isolated as the trihydrate of the p-*toluidine salt*; sinters at 100–110° and $[\alpha]_D$ −56° in water; Found: C, 49·05; H, 6·15; N, 6·0; H_2O, 10·9. $C_{13}H_{15}O_9N,C_7H_9N,3H_2O$ requires C, 49·0; H, 6·2; N, 5·7; H_2O, 11·0%).

5-Bromosalicylamide possessed one quarter to one half the tuberculostatic activity of T40 *in vitro* against strain $H_{37}R_v$ in Dubos medium.

REFERENCES

Urbanski, T. (1953). *Bull. Acad. Pol. Sci.* **1**, 319.
Urbanski, T., Hornung, S., Slopek, S. & Venulet, J. (1952). *Nature, Lond.*, **170**, 753.

Glucuronides of Salicylic Acid. By D. Robinson and R. T. Williams. (*Department of Biochemistry, St Mary's Hospital Medical School, London, W.* 2)

The occurrence of conjugated glucuronic acids in the urine of man and animals receiving salicylic acid has been known for a considerable time (Baldoni, 1905; Neuberg, 1911), but they have never been isolated. Two monoglucuronides are possible, namely, the ester, *o*-hydroxybenzoylglucuronide, and the ether, *o*-carboxyphenylglucuronide. Using ^{14}C-salicylic acid, Alpen, Mandel, Rodwell & Smith (1951) have demonstrated both forms in human urine, the ether glucuronide occurring in greater amounts than the ester. In dog urine, the glucuronide appears to be all of the ether type.

From the 24 hr. urine of a patient receiving 14 g. of salicylic acid a day, we have isolated, by the lead acetate separation, a glucuronide gum which, on methylation and acetylation, yielded 25 mg. of a derivative of the ester glucuronide of salicylic acid, which appears to be *methyl* (o-*acetoxybenzoyl tri*-O-*acetyl*-β-D-*glucosid*)*uronate*, m.p. 134–137° and $[\alpha]_D$ −30·5° in $CHCl_3$ (Found: C, 53·0; H, 5·2; OMe, 6·0. $C_{22}H_{24}O_{13}$ requires C, 53·2; H, 4·9; OMe, 6·25%). It was reducing and yielded salicylic acid on hydrolysis. The ether glucuronide was not isolated.

The experiment was repeated on rabbits, but no crystalline material was isolated. However, on feeding rabbits with methyl salicylate, a derivative of the ether glucuronide of salicylic acid was isolated similarly in minute yields. This appeared to be *methyl* (o-*carbomethoxyphenyl tri*-O-*acetyl* β-D-*glucosid*)*uronate*, m.p. 112° and $[\alpha]_D$ −40·7° in $CHCl_3$ (Found: C, 54·0; H, 5·2. $C_{21}H_{24}O_{12}$ requires C, 53·8; H, 5·2). This was non-reducing and yielded salicylic acid on hydrolysis. On treatment with alcoholic ammonia, it yielded what appears to be o-*carbomethoxyphenyl* β-D-*glucosiduronamide*, m.p. 196° and $[\alpha]_D$ −57·5° in aqueous ethanol (Found: C, 48·6; H, 5·7; N, 4·2. $C_{14}H_{17}O_8N,H_2O$ requires C, 48·7; H, 5·55; N, 4·1%).

Attempts to synthesize these compounds from biosynthetic *o*-methoxybenzoylglucuronide and salicylamide glucuronide have so far been unsuccessful.

REFERENCES

Alpen, E. L., Mandel, H. G., Rodwell, V. W. & Smith, P. K. (1951). *J. Pharmacol.* **102**, 105.
Baldoni, A. (1905). *Jber. Tierchem.* **35**, 124.
Neuberg, C. (1911). *Klin. Wschr.* **38**, 798.

α-Glucuronidase and Other Glycosidases from *Patella vulgata*. By J. Conchie, G. A. Levvy and C. A. Marsh. (*Rowett Research Institute, Bucksburn, Aberdeenshire*)

In connexion with the study of the selective inhibition of glycosidases by the corresponding aldonolactones (Conchie & Levvy, 1955), α- and β-mannosidase were required. Neither enzyme was present in the rumen of the sheep, but both were present in the common limpet (*Patella vulgata*). The enzyme preparation, precipitated between 20 and 80% saturation with ammonium sulphate, was also rich in the following: α- and β-glucosidase, α- and β-galactosidase, α- and β-*N*-acetylglucosaminidase, and β-xylosidase. In addition, as well as β-glucuronidase (Dodgson, Lewis & Spencer, 1953), an α-glucuronidase was present. This enzyme could be clearly distinguished from the β-glucuronidase. For example, it was not inhibited at all by saccharo-1:4-lactone, and it was quite stable at pH 2·75. There was also evidence for the presence of enzymes hydrolysing carboxymethylcellulose (G. N. Festenstein, private communication) and methyl α- and β-galacturonide.

Mannono-1:4-lactone powerfully inhibited α- and β-mannosidase, but had no effect on any of the other glycosidases studied in the limpet preparation. In general, the latter also were inhibited by the aldonolactones of corresponding configuration. However, some cross-effects were noted, and in the case of α-galactosidase the effect of galactono-1:4-lactone was small. The failure of saccharo-1:4-lactone to inhibit α-glucuronidase was in striking contrast to its action on β-glucuronidase from the limpet as from other sources. Boiled mucic acid solution similarly distinguished α- and β-galacturonidase.

The authors are indebted to Dr G. N. Bollenback for a specimen of phenyl α-glucuronide.

REFERENCES

Conchie, J. & Levvy, G. A. (1955). *Biochem. J.* **61**, xvi.
Dodgson, K. S., Lewis, J. I. M. & Spencer, B. (1953). *Biochem. J.* **55**, 253.

The Reactions of Glycine and DL-α-Alanine with D-Glucose. By S. Lewin and Z. Kosinski. (*Physical Chemistry Laboratory, South-West Essex Technical College, London, E.* 17)

The equilibrium constants for the reversible reaction of glycine and alanine with glucose have been given at 23° as 8·6 and 6·7 respectively by Katchalsky (1941), who interpreted his aerobic results in terms of reactions involving one molecule of the anion of the amino acid with one molecule of glucose. Later, Katchalsky & Sharon (1953) gave the respective values as 14·6 and 6·9 at 30°. Preliminary experiments, however (Lewin S., unpublished observations), suggested that the reactions were more complex.

We have examined these reactions systematically, using aerobic and anaerobic conditions, amino acid concentrations M/6 to M/60, glucose concentrations of 0·1 to 1·3M, and initial pH's of 4 to 9 over the temperature range of 20–50°. The methods employed were similar to those used by Lewin (1955, 1956) in the case of the histidine-glucose reaction.

A series of steady pH states, parallel to—but having higher pH depression values than—those observed in the histidine-glucose reaction, was noted. However, the pH depressions of the first steady pH state were found to be in accord with a mechanism involving the co-existence of one molecule of the anion of the amino acid with both one and two glucose molecules. Also, evidence for very small combination on the acid side of the isoelectric point, of glycine, was found which suggests some zwitterion-glucose reaction. Further, the reactions were found to be exothermic, the results being in disagreement with the data given by Katchalsky (1941) and Katchalsky & Sharon (1953).

The pH depressions, at constant glucose concentration, were found to increase a little with amino acid dilution at all temperatures examined, in the case of alanine, but in the case of glycine this effect was noticeable only at the higher temperatures.

At the lower temperatures hardly any difference was found between the anaerobic and aerobic pH depressions of the first steady pH state, but rise in temperature, accompanied by increase in glucose concentration at the later steady pH states resulted in the aerobic pH depressions having strikingly greater values than the anaerobic ones.

The 'browning reaction' was noted in the amino acid-glucose solutions, but unlike the pH depression values obtained, and unlike the browning in histidine-glucose solutions, the results could not be reproduced repeatedly.

REFERENCES

Katchalsky, A. (1941). *Biochem. J.* **35**, 1024.
Katchalsky, A. & Sharon, S. (1953). *Biochim. biophys. Acta*, **10**, 290.
Lewin, S. (1955). *Biochem. J.* **61**, xxv.
Lewin, S. (1956). *Biochem. J.* (In the Press.)

The Stimulation of Connective Tissue Formation by Carrageenin. By D. S. JACKSON. (*Rheumatism Research Centre, University of Manchester*)

It has been reported that carrageenin, a sulphated polygalactose, extracted from the seaweed *Chondrus crispus*, when injected subcutaneously into the abdominal region of the guinea pig, stimulates the formation of connective tissue in this area (Robertson & Schwartz, 1953). This has been confirmed, and a study made of the formation in this situation of three forms of collagen, viz. neutral salt-soluble collagen (N.C.) (Jackson & Fessler, 1955), citrate-soluble collagen (A.C.) (Tustanovskii, 1947) and insoluble collagen isolated as gelatin (I.C.).

Twenty-one guinea pigs (N.I.M.R. strain) were injected subcutaneously with 5 ml. of a 1% (w/v) solution of carrageenin in saline. The animals were sacrificed in groups of three at various time intervals, and the connective tissue formed dissected out, pooled, weighed and macerated. The tissue was extracted by a method essentially that of Harkness, Marko, Muir & Neuberger (1954), except that the first extraction was with 0·2M-NaCl (pH 7·4), the N.C. being isolated by precipitation with sodium chloride to a concentration of 18% (w/v), the resulting filtrate dialysed against distilled water for several days and freeze-dried. The collagen content of the various fractions isolated was measured by determining the hydroxyproline content by the method of Neuman & Logan (1950).

The amounts of the three collagen fractions have been calculated as a percentage of total extracted collagen and in mg./100 g. wet weight of tissue. The results show that N.C. is present in considerably larger amounts than A.C. in the early stages of growth. The amount of A.C. rises to a value greater than that of N.C. after 6 days. The amount of I.C. rises rapidly up to 7–8 days and then more slowly, reaching a maximum at about 14 days.

These results support the conclusions of Harkness *et al.* (1954) that N.C., and not A.C., is the true precursor of mature collagen.

A water-soluble, hydroxyproline-containing fraction was found in the freeze-dried material. The significance of this is not known, but it is being further investigated.

REFERENCES

Harkness, R. D., Marko, A. M., Muir, H. M. & Neuberger, A. (1954). *Biochem. J.* **56**, 558.

Jackson, D. S. & Fessler, J. H. (1955). *Nature, Lond.*, **176**, 69.

Neuman, R. E. & Logan, M. A. (1950). *J. biol. Chem.* **184**, 299.

Robertson, W. van B. & Schwartz, B. (1953). *J. biol. Chem.* **301**, 689.

Tustanovskii, A. A. (1947). *Biochemistry, Leningr.*, **12**, 285.

The Action of Periodate on ACTH. By H. B. F. DIXON. (*Department of Biochemistry, University of Cambridge*)

Geschwind & Li (1954) treated α-corticotropin with periodate to confirm the presence of an *N*-terminal serine residue, but they did not report the biological activity of the product. White (1955) removed *N*-terminal serine together with the adjacent tyrosine residue by the action of aminopeptidase; this treatment inactivated corticotropin A. Corticotropin A_1 has now been treated with periodate (0·01M) for 8 min. at pH 9·2 and the product isolated by dialysis against dilute acetic acid, using a collodion membrane (Adair, 1956), followed by chromatography (Dixon & Stack-Dunne, 1955). One main peak was found which was slightly retarded by the carboxylic ion-exchange resin, but much less so than corticotropin A_1. This peak was inactive (0–20% of the activity of corticotropin A_1) in an ACTH test (Sayers, Sayers & Woodbury, 1948). Melanophore-stimulating (MSH) activity, assayed in frogs, was unchanged.

The effect of periodate differs from that of peroxide, which produces a smaller fall of retention volume in ion-exchange chromatography (Dixon & Stack-Dunne, 1955), and whose action is reversed by heating with a thiol (Dedman, Farmer & Morris, 1955). On treatment with peroxide, corticotropin A_1 loses both ACTH and MSH activities, and regains both, together with its original retention volume (Dixon, 1955, 1956) when heated with a thiol. Peroxide-inactivated corticotropin A_1 after being heated with a thiol for a short time shows the two peaks on chromatography, one active and the other inactive in both tests (Dixon, 1956). This provides evidence that the ACTH and MSH activities are both intrinsic to the corticotropin A_1, although corticotropin has only about 1/150 of the MSH activity of purified melanophore-stimulating peptide (Porath, Roos, Landgrebe & Mitchell, 1955). Although both activities are apparently possessed by the same corticotropin molecule, and equally affected by peroxide, the periodate-labile group is essential only for the ACTH activity.

In all these reactions, and in the failure of KBH_4 to regenerate peroxide-inactivated material,

the MSH activity of purified melanophore-stimulating peptide behaved like that of corticotropin A_1. No chemical treatment has been found that will allow MSH assays to distinguish between these two hormones without physical separation.

I am grateful to Dr G. S. Adair, F.R.S., for the collodion membranes, to Professor F. W. Landgrebe for the melanophore-stimulating peptide, to Mr M. W. Rees for advice and discussion and to Miss M. B. Thomas for assistance.

REFERENCES

Adair, G. S. (1956). *Biochem. J.* (accompanying communication).
Dedman, M. L., Farmer, T. H. & Morris, C. J. O. R. (1955). *Biochem. J.* **59**, xii.
Dixon, H. B. F. (1955). *Biochim. biophys. Acta*, **18**, 599.
Dixon, H. B. F. (1956). *Biochim. biophys. Acta* (in the Press).
Dixon, H. B. F. & Stack-Dunne, M. P. (1955). *Biochem. J.* **61**, 483.
Geschwind, I. I. & Li, C. H. (1954). *Biochim. biophys. Acta*, **15**, 442.
Porath, J., Roos, P., Landgrebe, F. W. & Mitchell, G. M. (1955). *Biochim. biophys. Acta*, **17**, 598.
Sayers, M. A., Sayers, G. & Woodbury, L. A. (1948). *Endocrinology*, **42**, 379.
White, W. F. (1955). *J. Amer. chem. Soc.* **77**, 4691.

Modified Collodion Membranes Impermeable by Proteins with Comparatively Small Molecules. By G. S. Adair (introduced by H. B. F. Dixon). (*Physiological Laboratory, University of Cambridge*)

A modified type of collodion membrane has proved useful in recent work on pressure dialysis and measurements of osmotic pressures of proteins with comparatively small molecules. Consistent results were obtained with a protein derived from elastin, with a molecular weight as low as 5500 ± 100 (Partridge, Davis & Adair, 1955).

A membrane with a fairly high permeability was prepared first, by pouring several coats of a collodion solution over a rotating glass mould, as described by Adair (1925), Cole (1933) and Alexander & Johnson (1949). One solution contained 4 g. pyroxylin (I.C.I. no. HL120/170), 50 ml. anhydrous ether, 50 ml. absolute alcohol and 6 ml. ethylene glycol.

After the membrane had been mounted and tested, it was soaked for a few hours in an aqueous solution of glycerol. The concentration of glycerol required was estimated from measurements of initial and final permeabilities made in earlier experiments. The greater part of the glycerol solution left inside the membrane was removed by a pipette. The membrane was then connected to a glass bulb, filled with air under pressure (400–700 mm.) and left until the last drops of liquid on the internal surface had been pressed out. The external surface must be in contact with the solution of glycerol throughout this process. The liquid on the external surface was then wiped off as rapidly and as completely as possible.

The membrane was inverted, left to dry in air for a few hours and then disconnected from the bulb of compressed air. The final permeability was measured after the membrane had been soaked in water to remove glycerol. This final permeability is correlated with the initial permeability, the concentration of glycerol and the humidity.

In one experiment, the initial permeability for water was 0·095 ml. filtered/min., for a cylindrical membrane, diameter 1·13 cm., length 8 cm., area 28·4 $cm.^2$, under a pressure equal to 441 mm. Hg at 18°. The final permeability after soaking in 15% (v/v) glycerol and drying was 0·006 ml./min. A membrane soaked in 20% glycerol had an initial permeability of 0·093 and a final permeability of 0·024 ml./min.

REFERENCES

Adair, G. S. (1925). *Proc. Roy. Soc.* A, **108**, 627.
Alexander, A. E. & Johnson, P. (1949). *Colloid Science*, **1**, 166, **2**, Appendix. Oxford: Clarendon Press.
Cole, S. W. (1933). *Practical Physiological Chemistry*, 9th ed. p. 65. Cambridge: Heffer.
Partridge, S. M., Davis, H. F. & Adair, G. S. (1955). *Biochem. J.* **61**, 11.

The Effect of Papain on the Viscosity of Chondroitin Sulphate. By Helen Muir.* (*Medical Unit, St Mary's Hospital, London, W. 2*)

Relatively viscous preparations of chondroitin sulphate are obtained by extraction of cartilage with neutral 10% $CaCl_2$ (Blix & Snellman, 1945). Mathews (1955) has shown by light scattering that such preparations have a mol.wt. of 150 000 compared with 18 000–48 000 for material extracted under various alkaline conditions.

Chondroitin sulphate was extracted from laryngeal cartilage of 6-month-old pigs by the method of Blix & Snellman (1945) as modified by Mathews & Dorfman (1953), who purified it by precipitation with $Co(NH_3)_6Cl_3$. This material was noticeably

* Empire Rheumatism Council Fellow.

viscous, the relative viscosity of a 0·5% solution in water being 6·1 at 25°. It contained 26·3% hexosamine and 14·1% sulphate. Although some amino acids were shown to be present by paper chromatography, the material was heat stable and showed no loss of viscosity on heating at 100° for 30 min. Neither heating nor protein precipitants produced any flocculation. The ultraviolet absorption spectrum and paper chromatogram showed that aromatic amino acids were almost absent. Loss of viscosity was observed above pH 8·5 and became faster as the pH was raised. The viscosity fell to a final value of 1·5 for a 0·5% solution. Crystalline trypsin, chymotrypsin and pepsin produced little change of viscosity after 30 min. However, crystalline papain immediately reduced the viscosity, which reached a limiting value of 1·5 within 15 min. for a 0·5% solution. *p*-Chloromercuribenzoate completely inhibited this effect, which was not therefore due to contamination of papain by papaya lysozyme (Smith, Kimmel, Brown & Thompson, 1955). Furthermore, some dialysable hexosamine appeared after treating with papain and the behaviour on paper chromatograms also changed. 45% (v/v) *n*-Propanol/55%, 0·2M boric acid was used with Whatman no. 2 papers and the polysaccharide stained with toluidine blue. Papain-digested polysaccharide moved as a compact spot, $R_P = 0{\cdot}58$. Alkali-degraded polysaccharide behaved similarly. Untreated material remained at the origin, as did material treated with those proteolytic enzymes which did not produce changes of viscosity. Partially degraded polysaccharide showed both spots and also intermediate streaks.

It is suggested that perhaps some of the amino acids which are present may be part of a complex of large molecular weight consisting chiefly of polysaccharide cemented by small amounts of peptide or protein, which is destroyed by papain and probably also by alkali.

REFERENCES

Blix, G. & Snellman, O. (1945). *Ark. Kemi Min. Geol.* A, **19**, 32.
Mathews, M. B. (1955). *Fed. Proc.* **14**, 252.
Mathews, M. B. & Dorfman, A. (1953). *Arch. Biochem. Biophys.* **42**, 41.
Smith, E. L., Kimmel, J. R., Brown, D. M. & Thompson, E. O. (1955). *J. biol. Chem.* **215**, 67.

Pyridoxal Phosphate as a Coenzyme of 5-Hydroxytryptophan Decarboxylase. By JOYCE BUXTON and H. M. SINCLAIR. (*Laboratory of Human Nutrition, University of Oxford*)

Some evidence has been presented previously to suggest that pyridoxal phosphate may be a coenzyme of 5-hydroxytryptophan decarboxylase. Clark, Weissbach & Udenfriend (1954) showed that this enzyme was inhibited by carbonyl reagents, but the inhibition could not be reversed by excess of pyridoxal phosphate. Beiler & Martin (1954), using extracts of guinea-pig kidney, confirmed this and also demonstrated inhibition by chelating agents; they found that the enzyme was not inhibited by deoxypyridoxine phosphate. Both groups of workers concluded that the coenzyme was firmly bound.

Young hooded rats were fed a diet deficient in pyridoxine and their litter-mates served as pair-fed controls. All rats received 1·2 mg. deoxypyridoxine weekly by mouth and, in addition, the control animals received 0·1 mg. pyridoxine hydrochloride daily. The animals were killed by decapitation after 8–10 weeks, when those not receiving pyridoxin had reached an advanced stage of deficiency but the controls appeared normal; the kidneys were removed for estimation of 5-hydroxytryptophan decarboxylase by a modification of the chemical method of Clark *et al.* (1954).

The amount of 5-hydroxytryptamine (5-HT) formed by kidney tissue of pyridoxine-deficient rats during incubation with 5-hydroxytryptophan was significantly lower than (about one-quarter) that of the pair-fed controls. Addition *in vitro* of pyridoxal-5-phosphate to the tissue from the deficient rats increased the amount of 5-HT formed to a level approaching that found in the control animals. An increase, small in comparison, produced by pyridoxal phosphate in the amount of the 5-HT formed by the kidney extracts from control rats is probably attributable to the deoxypyridoxine fed to them.

It is concluded that pyridoxal phosphate is a coenzyme of kidney 5-hydroxytryptophan decarboxylase.

We are grateful to Messrs Sandoz Ltd for supplies of 5-hydroxytryptamine and to Dr G. J. Martin of the National Drug Company, Philadelphia, for 5-hydroxytryptophan. We thank Mr F. E. O'Connor for skilled technical assistance.

REFERENCES

Beiler, J. M. & Martin, G. J. (1954). *J. biol. Chem.* **211**, 39.
Clark, C. T., Weissbach, H. & Udenfriend, S. (1954). *J. biol. Chem.* **210**, 139.

The Effect of Urea on the Enzymic Activities of Trypsin and Chymotrypsin. By J. I. HARRIS.* (*Carlsberg Laboratorium, Copenhagen*)

The assumption that protein denaturation and enzymic activity are mutually exclusive is, at best, valid only if enzyme activity is measured under conditions which are not in themselves conducive to denaturation or to reversal of a previously established denaturation effect. The crucial significance of relating changes in enzymic activity to changes in one or more of the physical characteristics of the enzyme protein, measured in the same chemical environment, was emphasized when it was shown (Anfinsen *et al.* 1955) that urea–'denatured' ribonuclease retains catalytic function. These considerations have led to a reinvestigation of the recent report (Viswanatha & Liener, 1955) that trypsin exists in an active (i.e. 'native') stable form in the presence of 8·3M urea.

The viscosity of trypsin solutions (1% at pH 4·0, 20°) in increasing concentrations of urea have been determined, together with parallel measurements of enzyme (esterase) activity using α-*N*-toluenesulphonylarginine methyl ester dissolved in appropriate concentrations of urea at pH 7·8, 25°. The intrinsic viscosity of trypsin, which increases as a function of urea concentration, could be correlated with a corresponding decrease in enzyme activity; this suggests the existence of a mobile equilibrium between native active trypsin and unfolded inactive trypsin, which depends on urea concentration. Equilibrium is rapidly attained at 20°, is independent of trypsin concentration, and is completely reversible on dilution (cf. Northrop, 1932; Anson & Mirsky, 1933). In 8M urea trypsin exists entirely in a stable, unfolded, but inactive form, which regains its former active configuration when assayed by dilution under the conditions used by Visnawatha & Liener (1955). At intermediate concentrations of urea the initially formed reversible equilibrium states are subject to a secondary irreversible inactivation process which is maximal in 4M urea, where according to the equilibrium hypothesis a 1:1 ratio of active enzyme and potential substrate in the form of denatured enzyme is present. Kinetic considerations (as well as preliminary end-group studies) suggest that inactivation is due to an enzyme-catalysed autolysis which depends on trypsin concentration, pH and temperature (Harris & Linderstrøm-Lang, unpublished).

Similar results have been obtained with α-chymotrypsin; in this case, urea denaturation was not immediately reversed on dilution in the presence of substrate at pH 7·8.

Unlike ribonuclease, trypsin and α-chymotrypsin are not active in an unfolded form, which suggests that covalent cross-linkages alone are insufficient to maintain the integrity of the specific geometrical configurations which are essential for catalytic activity.

* Scientific Staff, Medical Research Council; present address, Department of Biochemistry, University of Cambridge.

REFERENCES

Anfinsen, C. B., Harrington, W. F., Hvidt, Aa, Linderstrøm-Lang, K., Ottesen, M. & Schellman, J. (1955). *Biochim. biophys. Acta*, **17**, 141.

Anson, M. L. & Mirsky, A. E. (1933). *J. gen. Physiol.* **17**, 393.

Northrop, J. H. (1932). *J. gen. Physiol.* **16**, 323.

Viswanatha, T. & Liener, I. E. (1955). *J. biol. Chem.* **215**, 777.

Deproteinization of Blood. By G. HUNTER. (*Stoke Mandeville Hospital, Aylesbury*)

The proteins of plasma, cells and whole blood, at the usual dilutions, e.g. 1 in 5 to 1 in 50, are quantitatively removed by appropriate adjustment of the H-ion concentration followed by heat precipitation and centrifugation or filtration. One litre of plasma, cells and whole blood require 50, 27 and 40 m-equiv. acetic acid to adjust the pH to about 5·3, 7·0 and 6·4 respectively, the points appropriate for precipitation by heat. The range of pH tolerance permitting water-clear filtrates, with the stated amounts of acid, suffices for nearly all bloods. The filtrates are comparable in appearance and in non-protein nitrogen to those obtained with tungstic or trichloroacetic acids; but being nearly neutral and containing little material added to the blood, they are suitable for various analytical procedures unsuited to filtrates made with the usual protein precipitants.

An explanation of the procedure, based on the isoelectric points of plasma and cell proteins and buffering capacity is offered.

Micro Determination of Magnesium in Serum with a New Titration Absorptiometer. By G. HUNTER (*Stoke Mandeville Hospital*) and F. D. STOTT (*M.R.C. Unit, Stoke Mandeville Hospital, Aylesbury*)

The colour change of Eriochrome Black T from blue to red at pH 10·2 has been used as a means of estimating Mg in plant materials by Gasser (1955) and in blood serum by Smith (1955). By the usual absorptiometer methods it is difficult to measure the amount of red form of the dye in presence of a varying and unknown excess of the blue form. In the present method this difficulty is overcome by use of the titration absorptiometer. An equal amount of a mixture of buffer and dye is placed in two tubes which are zeroed in the instrument with the light path down the axis of the fluid columns. To one tube the test portion containing Mg is added. There is a colour change toward red and deflexion of the pointer. Now to the other tube is added a standard solution of Mg until the pointer returns to zero. The amount of Mg added is the amount in the test portion. As the optical density is a measure of the total solute it is independent of the volume of solvent. And as this is a null method it is also independent of linearity in the concentration/density relation.

The serum must be freed from Ca and protein. As Smith has shown, trichloroacetate reduces the sensitivity of his method by about 20%. In the concentrations commonly used to estimate Ca in serum, oxalate also complexes the dye. Accordingly Ca is removed from the serum with a minimum of oxalate so that the amount in the subsequent test portion has a negligible complexing effect. The protein is removed from the Ca-free serum by the acid-heat method described in the previous communication. The amount of acetate added has likewise a negligible complexing effect.

Mg added to serum freed from Ca and Mg by filtration through an ion exchange resin is quantitatively recovered and replicate determinations are within 1%.

REFERENCES

Gasser, J. K. R. (1955). *Analyst*, **80**, 482.
Smith, A. J. (1955). *Biochem. J.* **60**, 522.

Isolation of Aromatic Substances Using Modified Charcoals. By A. ASATOOR and C. E. DALGLIESH. (*Postgraduate Medical School, Ducane Road, London, W.* 12)

Charcoal has frequently been used for the isolation of natural products, but in many cases adsorption is largely irreversible, and the adsorbate is recoverable in only poor yield. Pretreatment of the charcoal with a deactivating agent (e.g. stearic acid; Synge & Tiselius, 1949) blocks the most active sites on the charcoal surface and leaves available sites from which the adsorbate can be more readily displaced. Using charcoal deactivated with 4% by weight of stearic acid the aromatic metabolites in urine can be readily adsorbed (and thereby separated from salts and aliphatic substances) and the majority subsequently eluted with aqueous phenol (Dalgliesh, 1955).

A quantitative survey of the recovery of a wide range of aromatic substances has shown that the method works well with benzene derivatives (including phenols), indoles, purines and pyrimidines. Pyrroles and pyridine derivatives are less well recovered, whilst iminazole derivatives behave like aliphatic substances. Conjugates, e.g. with glycine, glucuronic acid and sulphuric acid, are in general recovered as satisfactorily as unconjugated substances.

Poorer recoveries are obtained with polycyclic substances, e.g. riboflavin, and substances carrying many hydrogen bonding groups on the aromatic ring. Recoveries of such substances can be improved by increasing the degree of deactivation of the charcoal. Poor recoveries are also obtained with basic substances such as tryptamine and adenine. Increasing the degree of stearic acid deactivation has comparatively little effect on recovery of basic substances using a neutral eluent, but high recoveries can be obtained using an acid eluent, such as phenol-acetic acid. This is not due to ion exchange involving the stearic carboxyl, as charcoals deactivated with octadecane or paraffin wax behave similarly.

Use of acid eluents is undesirable in, e.g., urine analysis, because of the increased risk of degradation of labile substances during subsequent concentration. However, basic substances can be readily displaced by a neutral eluent (aqueous phenol) if the charcoal has been deactivated with octadecylamine, which may prove in many ways preferable to stearic acid as a deactivating agent for general use. Surface-active agents can also bring about useful modifications in the adsorptive properties of charcoal.

Recoveries of a range of substances using series of variously modified charcoals under standardized conditions will be reported.

REFERENCES

Dalgliesh, C. E. (1955). *J. clin. Path.* **8**, 73.
Synge, R. L. M. & Tiselius, A. (1949). *Acta chem. scand.* **3**, 231.

Determination of Serum Protein-bound Iodine using an Alkaline Incineration Method. By J. D. ACLAND (introduced by A. JORDAN). (*Department of Pharmacology and Therapeutics, University of Sheffield and Sheffield Royal Infirmary*)

Determination of the protein-bound iodine (PBI) content of 1 ml. serum samples by methods employing alkaline incineration consists of three stages: (1) precipitation of serum protein, (2) conversion of organically bound iodine to iodide by ashing the precipitate with alkali, and (3) estimation of the eluted iodide by its catalytic action on the reduction of ceric salts by arsenious acid.

It has previously been reported (Acland, 1955) that, in the presence of zinc, the catalytic action of iodide is decreased and the relationship between iodide concentration and log E (measured at 415 mμ., 12·5 min. after starting the reaction) is no longer rectilinear. If serum proteins are precipitated by Somogyi's (1930) method, the acid eluents used by Barker, Humphrey & Soley (1951) and by Brown, Reingold & Samson (1953) completely dissolve zinc from the ashed protein precipitate, thereby making the construction of a straight-line calibration curve impossible. However, in the alkaline elution conditions of Grossman & Grossman's (1955) method, zinc remains in the residue and a straight-line calibration curve can be obtained.

Duplicate determinations on the same serum sample showed that considerable and variable losses occurred during the elution of iodide from ashed serum protein in the strongly acid conditions recommended by Barker *et al.* (1951) and by Brown *et al.* (1953). Consistent results were, however, obtained with the eluent recommended by Grossman & Grossman (1955).

Using an analytical procedure modified in accordance with the above findings, a series of 75 determinations on a serum sample gave a mean value of 5·82 μg. PBI/100 ml. with S.D. of $\pm$0·31 μg. (range 5·13–6·42). The mean recovery of 0·1 μg. I added as DL-thyroxine to 1 ml. of the same serum was 96·5% with S.D. (8) of $\pm$2·1% (range 93·5–99·8%).

I wish to thank Professor E. J. Wayne and Professor G. M. Wilson for their interest in this work, and the Board of Governors of the United Sheffield Hospitals for financial assistance.

REFERENCES

Acland, J. D. (1955). *J. Physiol.* **128**, 61 *P*.
Barker, S. B., Humphrey, N. I. & Soley, M. H. (1951). *J. clin. Invest.* **30**, 55.
Brown, H., Reingold, A. M. & Samson, N. (1953). *J. clin. Endocrin.* **13**, 1136.
Grossman, A. & Grossman, G. F. (1955). *J. clin. Endocrin.* **15**, 354.
Somogyi, M. (1930). *J. biol. Chem.* **85**, 655.

The Anomalous Kinetics of Arylsulphatase A of Human Tissues. By K. S. DODGSON and B. SPENCER. (*Department of Biochemistry, University of Wales, Cardiff*)

During studies on arylsulphatase A of human tissues (Dodgson, Spencer & Wynn, 1956), using nitrocatechol sulphate as substrate, the unusual enzyme concentration-activity curves which were described by Roy (1953) for the analogous enzyme of ox liver could be reproduced only when incubation periods of 60 min. were used. Using low concentrations of an enzyme preparation which was free from inorganic sulphate, time-activity curves showed an initial period (10–15 min., stage I) of rapid hydrolysis which was followed by a sharp decrease in the rate of hydrolysis (stage II). The decrease in activity was not maintained, however, and after about 60 min. the rate of hydrolysis again increased (stage III) although it was less than that observed in stage I. The irregular nature of the time-activity curve became less marked if the enzyme concentration was increased and at relatively high enzyme concentrations the curve approached a straight line although stage I was still apparent. The relationship between enzyme concentration and the rate of hydrolysis was only linear over short incubation periods (up to 10 min.) and when incubation was prolonged the relationship became complex.

In the presence of increasing concentrations of Na_2SO_4 (up to 0·00125M) the shape of the time-activity curve using low enzyme concentrations approached that obtained with high concentrations of enzyme in the absence of added sulphate, that is, stage II disappeared and the slope of stage III was greatly increased. Phosphate (up to 0·00125M) produced a similar effect. Other results obtained by adding sulphate or barium acetate to the incubation mixture at various times after the commencement of incubation support the view that inorganic sulphate, liberated from the substrate, is a major factor to be considered in explaining the anomalous curves. Other factors influencing the time-activity curves included the liberated nitrocatechol, substrate concentration, pH and temperature. High concentrations of substrate favoured

stage I, low concentrations favoured stage III. The effect of temperature was such that the rate of enzymic hydrolysis at 20° was initially much greater than at 37° but after several hours the two curves crossed.

A possible explanation of these observations will be discussed. Dr A. B. Roy (private communication) has been able to repeat many of our observations using ox arylsulphatase A, and it is now clear that the hypothesis originally proposed to explain the anomalous kinetics of this enzyme (Roy, 1953) is no longer tenable.

REFERENCES

Dodgson, K. S., Spencer, B. & Wynn, C. H. (1956). *Biochem. J.* (in the Press).
Roy, A. B. (1953). *Biochem. J.* **55**, 653.

The Preparation and Fractionation of Acidic Polysaccharides using Long-Chain Quaternary Ammonium Compounds. By J. E. Scott. (*Chemical Pathology Laboratory, Department of Pathology, University of Manchester*)

Long-chain quaternary ammonium salts such as cetylpyridinium chloride form very insoluble precipitates with acidic high polymers (Jones, 1953; Scott, 1955*a*, *b*). These precipitates are soluble in salt solutions. The concentration of salt required to dissolve the precipitate or to prevent precipitation in the presence of cetylpyridinium ions depends on the structure of the polyanion (Scott, 1955*b*).

It has been found that complete separation of several polyanions in solution can be achieved by precipitation with cetylpyridinium chloride at the appropriate concentration of inorganic salt. Hyaluronic acid is separable from chondroitin sulphate in 0·2M sodium sulphate. Experiments with chondroitin [^{35}S]sulphate and unlabelled hyaluronic acid showed that 100% of the radioactivity was recoverable, 97·5% being in the fraction insoluble in 0·2M sodium sulphate.

Cetylpyridinium and other quaternary ammonium ions can be removed from solution as already described (Scott, 1955*a*) or more simply by adsorption on fuller's earth. The solubility of long-chain detergents is greatly diminished below the critical solution temperature and many of them can be largely removed from solution by cooling. In the case of cetylpyridinium chloride, 5° is a sufficiently low temperature. This method is useful when additives are undesirable.

Alternatively, the polysaccharide may be removed from the solution containing cetylpyridinium ions and salt by precipitation with ethanol or acetone. A final treatment of the redissolved polysaccharide with fuller's earth removes residual cetylpyridinium, proteins, amino acids and pigments.

Hyaluronic acid and chondroitin sulphate have been prepared from skin and other tissues in very high yield by combining the above technique with papain digestion. Neutral polysaccharides are not precipitated by cetylpyridinium chloride, but are left in the supernatant.

Other separations have been achieved, for example carrageenin from chondroitin sulphate, and alginic acid from alginic acid sulphate. The fractionation is sharp, and can be applied to samples of 1 mg. or less. A wide choice of salts is available according to the general principles already described (Scott, 1955*b*).

The shorter the length of the quaternary ammonium carbon chain, the lower is the concentration of salt required to dissolve the corresponding polyanion complex. Below C_{12}, the complexes, especially of carboxylic compounds, become appreciably more soluble and recovery may not be complete at low concentration.

REFERENCES

Jones, A. S. (1953). *Biochim. biophys. Acta*, **10**, 607.
Scott, J. E. (1955*a*). *Chem. & Ind.* p. 168.
Scott, J. E. (1955*b*). *Biochim. biophys. Acta*, **18**, 428.

Evidence for Metallic Bonds Linking Deoxyribonucleic Acids to Proteins in Mammalian Tissues. By K. S. Kirby. (*Chester Beatty Research Institute, Institute of Cancer Research, The Royal Cancer Hospital, London, S.W.* 3)

When certain mammalian tissues were extracted by a two-phase water and phenol system and the resulting mixture centrifuged, all the ribonucleic acids (RNA) were extracted into the aqueous layer while the deoxyribonucleic acids (DNA) remained insoluble.

Replacement of the water by certain salt solutions, followed by extraction with phenol, brought about the release of DNA which now appeared to be soluble in the aqueous layer. The salts which produced this effect were KCN, NaF, KCNS, sodium salicylate, sodium diethyldithiocarbamate, cup-

ferron and sodium azide (generally 0·155M solutions). A considerable number of other compounds had no effect on the release of DNA. It seemed reasonable to assume that the above salts were removing metallic ions and, since these may have come from the cytoplasm, nuclei were isolated from rat liver by three different methods and treated with phenol and water in the presence and absence of the above complexing agents. No DNA was liberated in the absence of any of the salts and the DNA prepared from the nuclei with the aid of a complexing agent had a high viscosity and could be frozen dried to a white fibrous felt.

Phenol was essential to the reaction since no DNA was liberated if urea was used in the place of phenol and very little by addition of either *n*-butanol or guanidine hydrochloride.

Preliminary analyses of the metals present in the insoluble DNA-protein complex from rat liver indicated a relative increase in the amounts of iron and manganese over those present originally in the liver.

Evidence that metals can promote the absorption of DNA by an insoluble protein has been obtained by measuring the uptake of DNA by collagen which had been treated with various metals. Very little DNA was absorbed when K ions were present but absorption was promoted by Fe, Co, Cu, Mn and Zn. Since there were sufficient arginine and lysine groups to absorb all the DNA electrostatically it is probable that some of the absorption took place through a metallic bond between the carboxyl groups of the protein and the phosphate groups of the DNA.

The Influence of Diet on [^{14}C]Glycine Incorporation into the Ribonucleic Acid of Rat Liver.

By CATHERINE M. CLARK, D. J. NAISMITH and H. N. MUNRO. (*Department of Biochemistry, The University, Glasgow*)

In previous studies, Munro, Naismith & Wikramanayake (1953) found that the uptake of ^{32}P by rat-liver ribonucleic acid (RNA) is determined by energy intake, whereas the amount of RNA is related to protein intake. In the present experiments, the influence of protein intake has been explored further, using [^{14}C]glycine to label the purine nucleus.

Rats fed for a week on diets either adequate in protein or free from protein were injected with [2-^{14}C]glycine and ^{32}P and the livers excised after 3, 6 or 9 hr. Glycine was isolated for specific activity determinations as the dinitrophenyl derivative from the free amino acid pool, the liver protein, and, following degradation to glycine, from the adenine and guanine of RNA and of the perchloric acid-soluble fraction of liver. Uptake of [^{14}C]-glycine was related to the activity of glycine in the amino acid pool, and of ^{32}P in the ribonucleotides to the activity of inorganic phosphate.

In the post-absorptive state, rats on the protein-containing diet showed about half the uptake of ^{32}P into RNA obtained with rats on the protein-free diet. Uptake of [^{14}C]glycine by adenine and guanine was even more markedly depressed. Feeding casein just prior to injection caused immediate elevation of uptake to the level found with the protein-free diet.

Rats fasted after a diet containing protein show a reduction in the amount of liver RNA, and this suggests that the reduced uptake of ^{32}P and [^{14}C]glycine found in the post-absorptive state is due to dilution of the label by breakdown products of RNA. In support of this contention, uptake of [^{14}C]glycine by the acid-soluble adenine and guanine compounds of the liver was found to undergo changes during fasting and on refeeding protein similar to those found for RNA. Moreover, the reduction in [^{14}C]glycine incorporation during fasting was much greater for guanine than for adenine, a result which is consistent with dilution by RNA breakdown, since the smaller pool of guanine compounds would suffer greater dilution. The immediate reversal of the picture on administering protein implies cessation of this breakdown in response to an increased supply of amino acids.

It is concluded that the stability, and therefore amount, of liver RNA is intimately related to the intensity of protein synthesis in the liver.

REFERENCE

Munro, H. N., Naismith, D. J. & Wikramanayake, T. W. (1953). *Biochem. J.* **54**, 198.

Adenosinetriphosphate (ATP) in Blood Platelets. By G. V. R. Born. (*Nuffield Institute for Medical Research, University of Oxford*)

Recently it has been shown that mitochondria-like granules which can be obtained from the adrenal medulla contain not only most of the pressor amine activity of the gland but also very high concentrations of ATP (Hillarp, Nilsson & Högberg, 1955; Blaschko, Born, D'Iorio & Eade, 1956). This association of ATP with biologically active amines suggested a study of the ATP content of blood platelets, because the platelets of several species are known to contain histamine and 5-hydroxytryptamine, and probably also adrenaline, in considerable amounts (Humphrey & Jaques, 1954; Weil-Malherbe & Bone, 1954).

Extracts of platelets obtained from freshly drawn blood were assayed for ATP by the firefly luminescence method, and for histamine and 5-hydroxytryptamine by using the isolated guinea-pig ileum and the isolated rat uterus, respectively. It was found that platelets do indeed contain ATP in high concentrations. Pig platelets contained 4·1, 4·4 and 4·5 μmoles ATP/g. moist weight, or about 1·1% of the dry weight. This is about one-tenth of the concentration of ATP found in the adrenal medullary granules richest in pressor amines but about equal to that in striated muscle which, so far as is known, has a higher concentration of ATP than any other tissue. ATP concentrations in human platelets were 1·2 and 1·9 μmoles/g. moist weight.

The pig platelets which had 4·4 μmoles ATP/g. also contained 0·13 μmoles histamine and 0·49 μmoles 5-hydroxytryptamine/g. moist weight. Hence the molar ratio of ATP to histamine and 5-hydroxytryptamine together was 7·1. This contrasts with the situation in the adrenal medullary granules in which adrenaline is in approximately seven-fold excess over ATP, on a molar basis.

Pig platelets retained about 80% of their ATP and histamine in 0·12M sucrose solution but only 25–50% of both substances in 0·05M sucrose solution. Most of the ATP which was released from pig and human platelets into hypotonic solutions was found in the media after centrifugation.

REFERENCES

Blaschko, H., Born, G. V. R., D'Iorio, A. & Eade, N. R. (1956). *Biochem. J.* (in the Press).

Hillarp, N. A., Nilsson, B. & Högberg, B. (1955). *Nature, Lond.*, **176**, 1032.

Humphrey, J. H. & Jaques, R. (1954). *J. Physiol.* **124**, 305.

Weil-Malherbe, H. & Bone, A. D. (1954). *Nature, Lond.*, **174**, 557.

Estimation of Extracellular Fluid Volume Using ^{82}Br. By J. E. S. Bradley, D. Davidsson, I. MacIntyre* and A. Rapoport.† (*Postgraduate Medical School, Ducane Road, London, W.* 12)

Bromide has many advantages as an indicator of extracellular fluid (E.C.F.) volume. It is slowly excreted, is not metabolized, and can be given orally. If ^{82}Br is used measurement is simple.

The volume measured is almost exactly the same as the chloride space (Gamble *et al.* 1953), and that this considerably exceeds the inulin space (Deane, Ziff & Smith, 1952) is not so much evidence of cellular chloride content, as of incomplete diffusion of inulin (Cotlove, 1954). The space not permeated by inulin includes connective tissue (Nichols, Nichols, Weil & Wallace, 1953), and probably the water in the gut, which may be a considerable proportion of the body water (Cizek, 1954).

Twenty-five healthy adults were given a dose of Na^{82}Br by mouth. In six, the bromide space was estimated at 3, 4, 5 and 24 hr. after oral administration, and in nineteen at 24 and 72 hr., using a total body counter to allow for urinary loss (Veall, Fisher, Browne & Bradley, 1955). There was no significant expansion after 24 hr. (mean change +0·03 l.; S.E.M. 0·22), but there was a highly significant expansion between 4 and 24 hr. (mean change 1·18 l.; S.E.M. 0·55; $t = 2{\cdot}14$, $P < 0{\cdot}01$ for a 'one-tailed' test). This is in conflict with the conclusions of Cheek (1953). However, delay in absorption was shown not to be the cause by continued counting over the abdomen, using a scintillation counter. Further evidence of a true expansion was obtained from fourteen estimations of bromide space in one subject between 2 and 24 hr. after intravenous administration. There was a statistically significant expansion of 1 l. between 4 and 24 hr. Since significant differences occurred in rate of expansion in different subjects between 4 and 24 hr., it is not possible to correct precisely for this incomplete diffusion. An equilibration time of 24 hr. should be allowed.

In our series (13 males, 12 females, aged 17–41 years) there was a high correlation with height and weight. The best single correlation was with the latter.

* Drummond Fellow.

† Fellow of the R. S. McLaughlin Foundation of Canada.

The regression equations are as follows:

(1) E.C.F. in litres (y), weight in kilos (w):

$y = 6{\cdot}271 + 0{\cdot}155w$ (S.E. 1·097).

(2) Height in cm. (h):

$y = -24{\cdot}743 + 0{\cdot}241h$ (S.E. 1·157).

(3) Height and weight:

$y = -10{\cdot}248 + 0{\cdot}093w + 0{\cdot}121h$ (S.E. 0·994).

In these equations extracellular fluid volume is calculated from $\frac{^{82}\text{Br retained in body}}{\text{Plasma concn. of } ^{82}\text{Br}} \times 0{\cdot}9$.

The factor 0·9 corrects for plasma proteins and the Donnan effect.

REFERENCES

Cheek, D. B. (1953). *J. appl. Physiol.* **5**, 639.

Cizek, L. J. (1954). *Amer. J. Physiol.* **179**, 104.

Cotlove, E. (1954). *Amer. J. Physiol.* **176**, 3.

Deane, N., Ziff, M. & Smith, H. W. (1952). *J. clin. Invest.* **31**, 200.

Gamble, J. L. jun., Robertson, J. S., Hannigan, C. A., Foster, C. G. & Farr, L. E. (1953). *J. clin. Invest.* **32**, 483.

Nichols, G. jun., Nichols, W., Weil, W. B. & Wallace, W. M. (1953). *J. clin. Invest.* **32**, 1299.

Veall, N., Fisher, H. J., Browne, J. C. M. & Bradley, J. E. S. (1955). *Lancet*, i, 419.

Determination of Total Fat *in vivo* Using ^{85}Kr. By D. DAVIDSSON, I. MACINTYRE,* A. RAPOPORT† and J. E. S. BRADLEY. (*Postgraduate Medical School, Ducane Road, London, W.* 12)

The methods available for *in vivo* determination of total body fat are indirect. Two principal methods are used. In one the specific gravity of the body is determined by weighing under water (Behnke, Feen & William, 1942). In the other, total body water is measured (Pace & Rathbun, 1945). Both methods may be refined by the simultaneous estimation of extracellular fluid volume, but they still rest on two assumptions: that cell hydration is constant, and that the composition of the fat-free body solids is constant.

The doubtful validity of these assumptions in disease makes a direct estimation desirable. If the total uptake of a suitable fat-soluble substance is known, it should be possible to calculate body fat. Lesser, Blumberg & Steele (1952) used cyclopropane uptake in rats in this way. The requirements of a suitable substance other than a high fat:water solubility ratio are rapid diffusion and easy estimation. ^{85}Kr is theoretically satisfactory; preliminary experiments with human subjects, breathing ^{85}Kr in a closed circuit, showed that uptake was approximately proportional to estimates of body fat determined indirectly.

The solubility of Kr in oil, water and in fluids simulating extra- and intra-cellular fluids has been measured at various temperatures. The influence of protein on these solubilities has been determined, and studies made of Kr uptake by various tissues.

After validation in animals *in vivo*, it is hoped to apply these results to measurements in man.

* Drummond Fellow.

† Fellow of the R. S. McLaughlin Foundation of Canada.

REFERENCES

Behnke, A. R., Feen, B. G. & William, W. C. (1942). *J. Amer. med. Ass.* **118**, 495.

Lesser, G. T., Blumberg, A. G. & Steele, J. M. (1952). *Amer. J. Physiol.* **169**, 545.

Pace, N. & Rathbun, E. N. (1945). *J. biol. Chem.* **158**, 685.

Determination of Total Body Water Using Tritium. By J. E. S. BRADLEY, D. DAVIDSSON, I. MACINTYRE* and A. RAPOPORT.† (*Postgraduate Medical School, Ducane Road, London, W.* 12)

The substances available for the determination of total body water by the dilution principle are urea (McCance & Widdowson, 1951), antipyrine (Soberman *et al.* 1949), ^2H_2O (Schloerb *et al.* 1950), and ^3H_2O (Pace, Kline, Schachman & Harfenist, 1947).

The isotopic methods are theoretically preferable, but they usually require elaborate techniques of estimation. However, if a gas-flow counter of the type described by Garrow & Piper (1955) is used, ^{3}H may be estimated directly in plasma and urine without preliminary treatment. Total body water may then be measured in non-oedematous subjects by measuring the activity of a sample of urine passed between 4 and 5 hr. after an oral dose of 2 mc of ^3H_2O. Determination by this method in ten normal subjects agreed very closely with measurements using antipyrine.

* Drummond Fellow.

† Fellow of the R. S. McLaughlin Foundation of Canada.

REFERENCES

Garrow, J. & Piper, E. A. (1955). *Biochem. J.* **60**, 527.

McCance, R. A. & Widdowson, E. M. (1951). *Proc. Roy. Soc.* B, **138**, 115.

Pace, N., Kline, L., Schachman, H. K. & Harfenist, M. (1947). *J. biol. Chem.* **168**, 459.

Schloerb, P. R., Friis-Hansen, B. J., Edelman, I. S., Solomon, A. K. & Moore, F. D. (1950). *J. clin. Invest.* **29**, 1296.

Soberman, R., Brodie, B. B., Levy, B. B., Axelrod, J., Hollander, V. & Steele, J. M. (1949). *J. biol. Chem.* **179**, 31.

The Kinetics of Sulphatase A. By A. B. Roy. (*Department of Biochemistry, University of Edinburgh*)

Roy (1953*a*) described a method for the determination of aryl sulphatases using dipotassium 2-hydroxy-5-nitrophenyl sulphate (nitrocatechol sulphate) as substrate and demonstrated the occurrence in ox liver of two sulphatases capable of hydrolysing this substrate. One of these enzymes, sulphatase A, was shown to exhibit anomalous kinetics for the hydrolysis of nitrocatechol sulphate. The reaction velocity was not directly related to the enzyme concentration but apparently to the enzyme concentration raised to the power of $\frac{3}{2}$ (Roy, 1953*b*). An explanation of this anomaly was suggested on the assumption that the enzyme could exist in a number of polymeric forms. Recently Dodgson & Spencer (private communication) pointed out that this explanation was incorrect and that the fundamental anomaly was that the reaction was not of zero order at low enzyme concentrations.

Reinvestigation of this problem has confirmed these observations of Dodgson & Spencer. A study of the hydrolysis of potassium *p*-nitrophenyl sulphate by sulphatase A suggested that the kinetics of this reaction were normal. Unfortunately the low rate of this hydrolysis made the interpretation of these experiments difficult, but the results suggested that the anomaly in the hydrolysis of nitrocatechol sulphate might not be due to the enzyme but to the substrate. It has now been shown that the anomalous kinetics of the hydrolysis of nitrocatechol sulphate are caused by the presence of some impurity in normal preparations of this substance. Purified specimens of nitrocatechol sulphate have been prepared through the chloroform-soluble methylene blue salt which was reconverted to the sodium salt by treatment with Zeo-Carb 225 (Permutite Co. Ltd, London). With these purified preparations of nitrocatechol sulphate as substrate the kinetics of sulphatase A activity were normal, the reaction being of zero order at all enzyme concentrations studied and the reaction velocity being directly related to the enzyme concentration.

It is therefore concluded that the previously suggested explanation for the anomalous kinetics of sulphatase A is wrong and that the effect is due to the presence of some impurity in normal preparations of nitrocatechol sulphate. No suggestion of the nature of this contaminant has been obtained.

REFERENCES

Roy, A. B. (1953*a*). *Biochem. J.* **53**, 12.
Roy, A. B. (1953*b*). *Biochem. J.* **55**, 653.

Effects of Alkali on the A-Ring of Steroids. By D. Abelson and P. K. Bondy (introduced by C. E. Dalgliesh). (*Department of Medicine, Postgraduate Medical School, Ducane Road, London, W.* 12, *and Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut*)

It has previously been shown (Abelson & Bondy, 1955) that the NaOH fluorescence test for Δ^4-3-ketosteroids on paper chromatograms (Bush, 1952) may be applied to the quantitative determination of these compounds in solution. For this purpose the most convenient reagent-solvent combination was found to be potassium *tert.*-butoxide dissolved in *tert.*-butyl alcohol. Using the Farrand fluorometer 0·01 μg. of steroid could be detected in 0·5 ml. reagent.

It was noted that, in addition to exhibiting a yellow-orange fluorescence in ultraviolet light, solutions of Δ^4-3-ketosteroids in the butoxide reagent were yellow in colour. The absorption spectrum, determined with a Beckman DU spectrophotometer, showed maxima at 260 and 385 mμ. The latter peak was unchanged in position after standing for 2 hr. at room temperature, and appears to be characteristic of Δ^4-3-ketones. The only exception found was cholestane-3:6-dione, which also showed a peak at 385 mμ (and exhibited fluorescence). Other steroids tested either gave no appreciable absorption throughout the range 230–600 mμ., or showed an absorption maximum below 385 mμ., with negligible absorption at the latter wavelength. Oestradiol, oestrone and oestriol showed a maximum at 310 mμ., probably related to the presence of the phenolic grouping in ring A (Callow, 1936), and a second more intense maximum near 240 mμ.

Increase in base concentration resulted in more rapid colour development. The rate of colour development also varied with the steroid used. At a given time and base concentration the optical density at 385 mμ. varied with steroid concentration in accordance with Beer's law. The structure of the fluorescent yellow compound has not yet been determined. Both sodium and potassium derivatives fluoresce.

REFERENCES

Abelson, D. & Bondy, P. K. (1955). *Arch. Biochem. Biophys.* **57**, 208.
Bush, I. E. (1952). *Biochem. J.* **50**, 370.
Callow, R. K. (1936). *Biochem. J.* **30**, 907.

DEMONSTRATIONS

A Titration Absorptiometer. By F. D. Stott (introduced by G. Hunter). (*Stoke Mandeville Hospital, Aylesbury*)

This instrument is designed to overcome some of the difficulties experienced in normal absorptiometric methods where coloured reagents are used. In principle, it is a photoelectric instrument for determining the point at which two solutions are identical in colour and optical density. Two sample cells are used, and the light is passed vertically down the axis of these cells on to a pair of barrier layer photocells, which are series connected to a microammeter incorporating a transistor amplifier.

By passing the light beam down the axis of the sample cells, the total optical density is measured independently of dilution by colourless solvents. Thus it is possible to carry out a titration in the instrument until an optical balance between the two cells is achieved, since this balance point is not affected by the volume change during the course of the titration. Provision is made to agitate the cells without removing them from the instrument, and the standard solution used in the titration can be run in from the burette without interrupting the observation of the colour change.

The instrument is demonstrated measuring magnesium concentrations at the μg./ml. level, using Eriochrome Black as an indicator.

Separation of Proteins by Electrodialysis through Filter-Paper 'Membranes'. By D. J. R. Laurence. (*Postgraduate Medical School, Ducane Road, London, W.* 12)

The electrodialysis cell is of five compartments separated by membranes which are horizontal during operation of the cell. The end two of the five compartments are electrode chambers with side arms for buffer flow, electrical contact being made via platinum electrodes sealed into the constant level outlets from the chambers. The central compartment is for the sample and between the sample compartments and the electrode chambers are compartments for receiving the fractions (upper and lower receivers). The compartments are made from Perspex blocks 6 cm. square in cross-section, each with a central hole 3 cm. in diameter, and are assembled face to face using threaded rod insulated with Polythene sleeves, and wing nuts. The sample compartment and receivers are 0·6 cm. in length and each has a hole in the side, 0·4 cm. in diameter, for filling and emptying the compartments. Cellophan membranes separate the receivers from the electrode chambers and the sample compartment and receivers are separated by sheets of Whatman no. 50 filter paper. Flow laterally through the filter paper is prevented by impregnating the paper with a ring of paraffin, the Perspex faces of the cells being lightly smeared with grease.

The receivers are filled first, the upper with buffer solution and the lower with buffer containing 15% glycerol. The protein sample dialysed against buffer is then introduced into the central compartment, the compartments being sealed with plugs and the apparatus placed in its working position as soon as possible. Buffer is run through the end compartments in a slow stream and the electrical connexions are made. The proteins migrate into upper or lower receivers depending on the resultant of electrophoretic and electrosmotic velocities.

With a current of 40 mA an albumin fraction stained with bromophenol blue in 0·05 M phosphate buffer pH 6·0 migrated from the sample compartment to the lower receiver in 7 hr. The descending boundary remained sharp and the cell temperature rose less than 10° above the surrounding temperature.

Svennson (1948) has reviewed similar types of apparatus.

REFERENCE

Svennson, H. (1948). *Advanc. Protein Chem.* **4**, 251.

Medical Research Council Steroid Reference Collection. By Sheila Ridley and W. Klyne. (*Postgraduate Medical School, London, W.* 12)

The collection supplies reference samples of steroids, particularly hormones and their metabolites, as standards for paper chromatography, colour reactions, infrared measurement, and mixed melting points—also, in some cases, as standards for methods of determination in urine and blood. A similar scheme has been organized by the U.S. Pharmacopoeia.

Exhibits will show the scope of the Collection. Research schemes involving systematic studies of complete series of isomers, or of compounds containing single functional groups, will be illustrated.

Generous gifts of steroids from many industrial firms are gratefully acknowledged.

Estimation of Antipyrine in Plasma. By D. DAVIDSSON and I. MACINTYRE.* (*Postgraduate Medical School, Ducane Road, London, W. 12*)

Brodie, Axelrod, Soberman & Levy (1949) described a method which has been widely used. This depended on the formation of 4-nitrosoantipyrine by the addition of $NaNO_2$ and H_2SO_4 to a plasma filtrate. Optical density at 350 mμ. was determined before and after the addition of $NaNO_2$, the increase at 20 min. being a maximum, and a measure of antipyrine content.

Ikkos *et al.* (1954), however, were unable to obtain consistent results without taking multiple readings and using the maximum for calculation. Kraybill, Goode, Robertson & Sloane (1954) found that 50 min. was required for the maximum extinction to be reached.

We have found that the laborious procedure of Ikkos *et al.* is unnecessary, if temperature is maintained at 22° after the addition of nitrite. A further simplification is possible if plasma, obtained before antipyrine administration, is available. In this case the optical densities need not be measured before the addition of $NaNO_2$.

Reagents

(1) Zinc reagent. 100 g. $ZnSO_4.7H_2O$ are dissolved in distilled water, 40 ml. 6N-H_2SO_4 are added and the solution made to 1 l.

(2) 0·75N-NaOH.

(3) 4N-H_2SO_4.

(4) $NaNO_2$; 0·2 g./100 ml.

(5) Antipyrine standards, containing 8 and 12 mg./l.

Procedure

Add 2 ml. plasma to 2 ml. distilled water in a centrifuge tube. Add 2 ml. zinc reagent. Mix. Add 2 ml. 0·75N-NaOH. Shake. After 15 min. centrifuge and then filter the supernatant. Place 3 ml. of the clear filtrate and 3 ml. of each standard in a test tube in a water bath at 22°. After 5 min. add 0·05 ml. 4N-H_2SO_4 and 0·1 ml. $NaNO_2$ solution. Allow the tubes to stand in the water bath for 25 min. Read the optical densities at 350 mμ. against a reagent blank of distilled water, H_2SO_4 and $NaNO_2$. The readings obtained are stable for 20 min. The standard concentrations are appropriate for the levels obtained in man after the intravenous administration of 2 g. antipyrine for estimation of total body water.

* Drummond Fellow.

REFERENCES

Brodie, B. B., Axelrod, J., Soberman, R. & Levy, B. B. (1949). *J. biol. Chem.* **179**, 25.

Ikkos, D., Luft, R. & Sjögren, B. (1954). *J. clin. Invest.* **33**, 989.

Kraybill, H. F., Goode, E. R., Robertson, R. S. B. & Sloane, H. S. (1954). *J. appl. Physiol.* **6**, 27.

Estimation of Calcium in Biological Fluids with Ethylenediamine Tetra-Acetic Acid. By D. DAVIDSSON (introduced by I. MACINTYRE). (*Postgraduate Medical School, Ducane Road, London, W. 12*)

The following procedure avoids the losses of calcium consequent upon washing the oxalate precipitate (MacIntyre, 1955) and provides a better end-point than the permanganate titration.

Reagents

(1) Ammonium oxalate 4 g./100 ml.

(2) 0·2N-HCl.

(3) 0·2N-NaOH.

(4) Indicator solution: 50 mg. of Solochrome Black W.D.F.A. (I.C.I.) are dissolved in 25 ml. absolute ethanol. This solution lasts for 2 days if kept in the refrigerator.

(5) Magnesium sulphate solution: 2 m-equiv./l.

(6) Buffer and indicator solution: to 10 ml. of ethanolamine are added 2 ml. indicator solution and 2 ml. of magnesium solution. This is then made to 100 ml. with distilled water.

(7) Di-sodium ethylenediamine tetra-acetate solution: 0·75 g. are dissolved in water and made up to 1 l.

(8) Stock calcium solution: 2·5022 g. of AnalaR $CaCO_3$, dried at 500°, are dissolved in 55 ml. N-HCl and made to 1 l. with distilled water.

(9) Dilute calcium standard: a standard of 5 m-equiv./l. is prepared by ten-fold dilution of the stock.

Procedure

Serum. Add 1 ml. distilled water to 1 ml. fresh serum in a centrifuge tube. Add 1 ml. ammonium oxalate solution. Mix. Allow to stand for 30 min. Centrifuge, pour off the supernatant and leave inverted tubes to drain on filter paper for 5 min. Dissolve the precipitate by blowing in 2 ml. of 0·2N-HCl and heating in a boiling-water bath. Pour the solution into a test tube. Wash the centrifuge tube with 5 ml. distilled water and allow the solution to cool. Add 3 ml. of buffer-indicator

solution. Titrate with di-sodium ethylenediamine tetra-acetate to a pure blue end-point. The calcium standard is prepared by adding 4 ml. distilled water, 3 ml. buffer-indicator and 2 ml. of 0·2 N-HCl to 1 ml. of dilute calcium standard. The reagent blank consists of the same solutions omitting only the dilute calcium standard.

Urine. Urine for calcium estimations should be collected in acid. Add 2–3 drops of bromphenol blue followed by 1 ml. of oxalate solution to 1 ml. urine. If the indicator is yellow, add 0·2 N-NaOH drop-wise until it is blue. Proceed as for serum.

REFERENCE

MacIntyre, I. (1955). *Rec. Trav. chim. Pays-Bas*, **74**, 498.

An Ultraviolet Flame Photometer. By I. MacIntyre* and J. E. S. Bradley. (*Postgraduate Medical School, Ducane Road, London, W.* 12)

Magnesium is not usually determined in body fluids by flame photometry. This is because its emissions at 280 and 385 mμ. are of low intensity and also outside the range of most instruments.

A flame spectrophotometer has been constructed to overcome these difficulties. The burner and sprayer units are substantially those described elsewhere (MacIntyre, 1956). An air-acetylene flame is used and the desired wavelength isolated with a Hilger D222 quartz monochromator. An E.H.T. unit operates an E.M.I. photomultiplier sensitive in the ultraviolet (Type 6256). A Pye 'Scalamp' galvanometer (sensitivity 180 mm./μamp.) measures the photomultiplier current.

A simple two-valve circuit provides a variable integrating time. This increases the precision of reading at levels of low light intensity.

The integrating condensers are kept charged to approximately the correct potential, so that the time taken to reach a steady reading is not greatly increased with longer integration times. This instrument has been used for the determination of calcium. Methods for analysis of magnesium, iron, and copper in biological fluids, using this instrument, are being developed.

* Drummond Fellow.

REFERENCE

MacIntyre, I. (1956). *Biochem. J.* (in the Press).

Some Improved Methods of Clinical Biochemical Analysis. By J. C. B. Fenton, J. Pryce and I. D. P. Wootton. (*Postgraduate Medical School, Ducane Road, London, W.* 12)

Titrimetric Determination of Aluminium with Ethylenediaminetetra-acetic Acid. By Rachel M. Paul (introduced by E. J. King). (*Postgraduate Medical School, Ducane Road, London, W.* 12)

This method is an adaptation of that of Theis (1955) in which Al is titrated directly at pH 4 at 80° with 0·05 M EDTA using 0·1 % Chromazurol S as indicator. At pH 4 this substance gives an orange colour, and in the presence of Al, a violet colour. It is necessary to carry out the titration very slowly as the initial formation of the complex is very slow. The end-point is best observed with a bright diffuse light such as is obtained with an X-ray viewing screen.

The Al-containing solution (0·2–10 mg. Al) is heated to 80° with 150 ml. acetate buffer of pH 4, and 3 drops of 0·1 % Chromazurol S indicator. The EDTA is added from a micro-burette. It was found best to add a small amount, e.g., 0·1 ml. 0·05 M EDTA, and heat for 5 min. before titrating. One ml. 0·05 M EDTA solution is equivalent to 1·345 mg. Al. With practice the end-point is clearly discernible when read in appropriate light and compared with a flask containing the reagent blank. For amounts of Al less than 0·5 mg. it is preferable to use 0·005 M EDTA. Ferric iron, which interferes, can be removed by alkaline precipitation with NaOH, or reduced from Fe^{3+} to Fe^{2+} with hydrazine sulphate, when the Fe^{2+} does not react with EDTA at pH 4.

This method was found to be much more rapid than the colorimetric hydroxyquinoline method of Gentry & Sherrington (1946) and gave equally satisfactory results.

REFERENCES

Gentry, C. H. R. & Sherrington, L. G. (1946). *Analyst*, **71**, 432.

Theis, M. (1955). *Z. anal. Chem.* **144**, 106.

An Apparatus for the Biosynthesis of [^{14}C]*Chlorella*. By R. W. Dutton. (*Postgraduate Medical School, Ducane Road, London, W.* 12)

Experimental Pneumoconiosis. By M. Yoganathan and F. M. Englebrecht (introduced by E. J. King). (*Postgraduate Medical School, Ducane Road, London, W.* 12)

[*The Editors of the Biochemical Journal accept no responsibility for the Reports of the Proceedings of the Society. Abstracts of papers read are published as received from the authors.*]

PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **348th Meeting** *of the Biochemical Society was held at the London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.* **1**, *on Saturday,* 18 *February* 1956, *at* 11 *a.m., and took the form of a Symposium on 'The Structure of Nucleic Acids and their Role in Protein Synthesis'.*

COMMUNICATIONS

Recent Views on the Chemical Structure of the Polynucleotides. By R. MARKHAM. (*Agricultural Research Council Virus Research Unit, Molteno Institute, Cambridge*)

Although the nucleic acids have now been known for more than 80 years, it is only quite recently that their biological importance and their extreme complexity has been fully appreciated. The main reason for this change in our ideas about nucleic acids is that accurate and highly sensitive methods for the analysis of these compounds have been developed and applied to carefully prepared specimens of nucleic acids obtained from very diverse sources. Until this had been done it had appeared that there were only two kinds of nucleic acid, typified by that from the mammalian thymus and that from yeast. The former, deoxyribonucleic acid, contained 2-deoxyribose, phosphoric acid and the heterocyclic bases adenine, guanine, cytosine and thymine (5-methyluracil), while the latter, ribonucleic acid, differed in having uracil instead of thymine, and ribose instead of 2-deoxyribose. Furthermore, analysis suggested that the bases occurred in equivalent amounts and it was generally assumed that the molecules had each four phosphorus atoms, four sugar residues and one of each of the four bases, and so had a molecular weight of about 1200. Substances of this type could, of course, have very little biological specificity.

Recent analysis has shown, however, that at least three more bases occur naturally, namely 5-methylcytosine, 5-hydroxymethylcytosine (glucoside) and 6-methylaminopurine, all being present in one or more deoxyribonucleic acids. Moreover, the proportions of the bases vary to some extent in deoxyribonucleic acids, while the variation in the bases in various ribonucleic acids is very considerable indeed.

Largely due to the difference in the sugar part of the molecule, ribonucleic acids have proved to be much more amenable to analysis than have deoxyribonucleic acids. Methods have been developed for the isolation and characterization of small fragments of the molecules and for the determination of the nature and number of the terminal residues at each end of the chain. A method is also available for step-by-step degradation, and it would now appear that the only serious obstacle to the determination of the complete nucleotide sequence in a ribonucleic acid is the fact that these substances occur as complex mixtures of molecules of different compositions, even in the small plant viruses which would seem to be the most hopeful sources of uniform nucleic acids.

Deoxyribonucleic acids present a much more difficult problem, even though they are usually obtained from sources which are more or less inert metabolically. On the whole they seem to be much larger than the ribonucleic acids and while a number of small fragments have been obtained by enzymic and chemical hydrolysis very little is known about nucleotide sequences. Possibly the most significant single piece of information about these nucleic acids is the observation that there is a tendency for pairs of bases to occur in equivalent proportions. Thus, in many deoxyribonucleic acids, adenine occurs in an amount nearly equivalent to thymine, and guanine to cytosine. This relationship has formed the basis of much speculation about the gross molecular structure of deoxyribonucleic acids, but our understanding of the chemistry of these substances is not sufficiently advanced for us to do anything other than point out minor discrepancies or agreements between experimental fact and pure hypothesis.

The general picture which one now has of the nucleic acids is of linear structures composed of nucleoside residues joined by phosphodiester links between C′3 and C′5, whatever specificity the molecule has being decided by the arrangement of the bases along the chain, which may be quite short in the ribonucleic acids, but may be many hundreds of residues long in the deoxyribonucleic acids.

Structure of Deoxyribosenucleic Acid and Nucleoprotein and its Possible Bearing on Protein Synthesis. By M. H. F. WILKINS. (*Medical Research Council Biophysics Research Unit, King's College, London, W.C.* 2)

The precise dimensions of the deoxyribosenucleic acid molecule may be determined by measuring the intensities on the X-ray diffraction photograph and by building accurate molecular models. The Fourier transform of the model is calculated and the model is adjusted until the transform corresponds with the observed X-ray intensities. The dimensions of the molecule are not very different from those of the model built by Watson & Crick; e.g. P atoms lie on a radius of ~9Å instead of 10Å. There are two helical grooves on the surface of the molecule, the nitrogen bases lying at the bottom of the grooves. In sperm heads the protamine molecule is wound around the shallower of the two grooves, but histone *in vivo*, although combined with the nucleic acid, is not attached in the same way as protamine. Non-basic residues in protamine occur in pairs in the polypeptide chain and probably form loops in the chain. Lysine-rich histone can combine *in vitro* with DNA in the same way. A characteristic X-ray diffraction picture is given by nucleohistone in intact calf thymus and fowl erythrocyte nuclei, sea-urchin sperm heads, and water-extracted thymus nucleohistone. Evidently the structure remains substantially intact during extraction. Although these observations give information about how protein binds to DNA, their implications with regard to protein synthesis are not immediately clear except to show that some proposed schemes are obviously incorrect and others have serious weaknesses. Ribonucleic acid does not give such well-defined X-ray diffraction pictures as DNA and hence progress in the elucidation of its structure has been more slow.

Nucleic Acids and Protein Synthesis in Animal Tissues. By BRIGITTE A. ASKONAS, J. L. SIMKIN and T. S. WORK. (*National Institute for Medical Research, London, N.W.* 7)

It is well known that cells concerned with rapid growth and protein synthesis contain large amounts of ribonucleic acid (RNA). This has led to the view that RNA is connected with the synthesis of proteins. In recent years, evidence supporting this view has been obtained by a number of investigators and their findings will be discussed.

The problem has been studied by injecting radioactive amino acids into animals and determining the distribution of radioactivity in the subcellular protein fractions. It was found that the protein of the microsome fraction became labelled more rapidly than other cell proteins. Further fractionation of the microsome material has shown maximum labelling in ribonucleoprotein fractions of the microsomes. Thus protein associated with RNA appears to turn over more rapidly than other cellular proteins.

In addition to experiments with whole animals, various investigators have studied protein synthesis by incubation of tissue slices, homogenates and isolated subcellular fractions. It is possible to follow synthesis of a specific protein in tissue slices by the use of immunological or enzymic methods and by techniques using radioactive amino acid. With one exception so far, it has not proved possible to obtain synthesis of a specific protein after the cells have been broken. With homogenates, experiments have been based mainly on measuring incorporation of radioactive amino acids into undefined proteins of the different cellular fractions. Here also, the maximal activity after incubation with radioactive amino acids has been found in nucleoprotein fractions. The incorporation process is dependent on the presence of energy sources and on undegraded RNA in microsomes or DNA in nuclei. The significance of these results will be discussed.

Nucleic Acids and the Incorporation of Amino Acids. By E. F. GALE. (*Medical Research Council Unit for Chemical Microbiology, Department of Biochemistry, University of Cambridge*)

Incubation of cell and tissue preparations in the presence of a labelled amino acid frequently results in incorporation of the labelled residue into proteins of the preparations. If protein synthesis occurs during the incubation, then the labelled amino acid becomes part of the newly formed protein. Incorporation also occurs under conditions in which no protein synthesis can be demonstrated, while studies of the rate of incorporation of any specific amino acid in the presence of various inhibitors show that this rate cannot be correlated with the rate of net protein synthesis. Thus a strain of *Staphylococcus aureus* requiring 14 amino acids for growth or protein synthesis will incorporate glutamic acid or glycine (or other amino acid) when incubated with that amino acid alone;

further, incorporation of glutamic acid is unaffected by the presence of *p*-chlorophenylalanine, although this substance inhibits the incorporation of phenylalanine and the net synthesis of protein. Incorporation, occurring in the absence of net protein synthesis, may take place by a process simpler than, or forming part of the mechanism of, that involved in total protein synthesis.

Incorporation in fragmented bacterial or mammalian cell preparations requires a source of energy which can be supplied by ATP. In disrupted staphylococcal cells incorporation of glycine or glutamic acid is inhibited by dinitrophenol, penicillin, bacitracin, chloramphenicol or 8-hydroxyquinoline. Removal of nucleic acid from disrupted staphylococci results in a decrease in the rate and amount of incorporation that will take place; a given degree of nucleic acid depletion affects incorporation of different amino acids to different extents. Incorporation can be restored by addition of staphylococcal nucleic acid to the incubation mixture; DNA gives complete restoration whereas RNA is less effective. Treatment with deoxyribonuclease decreases the activity of DNA, while ribonuclease digestion of RNA results in increased activity, the active material becoming dialysable. Fractionation of such RNA digests by chromatographic and ionophoretic procedures has led to the separation of a number of 'incorporation factors' of similar physico-chemical properties but differing in the spectrum of amino acids whose incorporation they promote. Certain factors were earlier identified tentatively as small polynucleotides; further purification has resulted in separation of activating substances from the polynucleotide fractions and these substances appear to be non-nucleotide in nature. A 'Glycine Incorporation Factor' has been isolated which is soluble in ethanol, insoluble in ether, and stable at 100° for 1 hr. in either N-HCl or N-NaOH but slowly destroyed on further heating.

Interrelationships of Nucleic Acid and Protein in the Multiplication of Bacteriophage. By K. Burton. (*Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry, University of Oxford*)

The paper will be connected with *Escherichia coli* and bacteriophage T2 or the closely related T4 and T6 phages. The structure of the phage particles and their multiplication will be reviewed in the light of the evidence which has been obtained by chemical, genetic and radiobiological methods.

The deoxyribonucleic acid (DNA) is virtually the only component of the phage particle which enters the host cell. Ribonucleic acid is not a constituent of bacteriophages and does not appear to be involved in the multiplication of, at least, the even-numbered T phages. Almost all of the protein and most of the DNA of the progeny particles is synthesized from the constituents of the growth medium after the infection of the host cell. The phage particles are devoid of detectable metabolic activity and the necessary biosyntheses must therefore be performed by the metabolic machinery of the host cell.

The synthesis of DNA containing 5-hydroxymethylcytosine commences within 3 min. after infection. Hydroxymethylcytosine is characteristic of phage DNA and is not present in bacterial DNA. The phage-type DNA is at first formed from degradation products of the bacterial DNA but after 6–10 min. it is synthesized from the constituents of the medium. Kinetic tracer studies of the synthesis of protein and of DNA have demonstrated that there are separate pools of precursor DNA and precursor protein from which the mature phage particles are assembled at random. The protein of the progeny phage particles is, on the average, synthesized later than the DNA. The initiation of the synthesis of phage DNA requires a synthesis of protein in the first stages after infection. Once initiated, the synthesis may continue in the absence of protein synthesis but the DNA is usually synthesized at a faster rate if the protein synthesis is allowed to continue.

The distribution of spontaneous phage mutants among the progeny of different infected cells agrees very closely with the hypothesis that the phage genes multiply by unsynchronized exponential doubling. There is much evidence that the genetic specificity is carried by the DNA of the resting phage particle and there is also evidence to indicate that, during the multiplication process, the precursor DNA carries the genetic specificity that ultimately appears in the mature progeny phage. Taken with the fact that phage DNA can be synthesized in the absence of protein synthesis, these hypotheses of the mode of gene multiplication and of the genetic properties of the DNA suggest that a protein template is not involved in the synthesis of the phage DNA. This conclusion must be considered very cautiously until there is further information, principally about the function of the protein that must be synthesized in order to initiate the synthesis of the phage DNA; certain important radiobiological findings would also require fuller explanation.

Radio-iodinated Human Serum Albumin

Regular weekly supplies of human serum albumin labelled with iodine-131 are now available to hospitals and research institutions.

Iodinated human serum albumin has been employed in a variety of diagnostic procedures. It is at present most widely used in the determination of blood volumes and other studies of circulation.

Further details of this material will gladly be supplied upon request.

THE RADIOCHEMICAL CENTRE, AMERSHAM, BUCKINGHAMSHIRE, ENGLAND

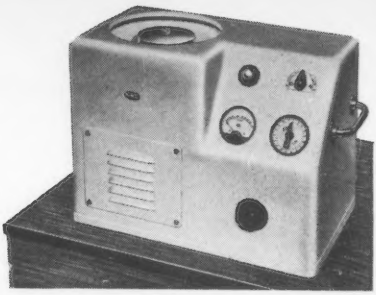
TAS/RCII

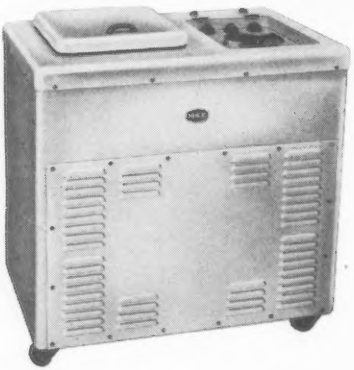
MSE

HIGH-SPEED 13 CENTRIFUGE

13,000 R.P.M.
20,000 x G.

50 ml 7 ml 5 ml
TUBE SIZE





WITH OR WITHOUT REFRIGERATION

13,000 r.p.m. 400 ml capacity. Available as a bench model without refrigeration or as a mobile floor model with self-contained refrigeration for temperatures down to −15°C. at full centrifuging speeds. There is a choice of two heads—a totally enclosed swing-out type head of special MSE design carries eight 5 ml stainless steel containers, and an angle head of dual capacity; it will carry either 8 × 50 ml tubes, or using special adaptors 8 × 7 ml tubes.

Ask for MSE Bulletin No. 140

MEASURING & SCIENTIFIC EQUIPMENT LTD., Spenser Street, London, S.W. 1 · Victoria 5426

AUSTRALIA

UNIVERSITY OF SYDNEY

Applications are invited for the position of *Lecturer in Biochemistry*.

The salary for a Lecturer is within the range £A1200/£A1750 per annum, plus cost of living adjustment (£A31 men, £A23 women), and with annual increments of £A80. The salary is subject to deductions under the State Superannuation Act. The commencing salary will be fixed according to the qualifications and experience of the successful applicant.

Finance available for home purchase under Staff Members' Housing Scheme.

Further particulars and information as to the method of application may be obtained from the Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C. 1.

The closing date for the receipt of applications, in Australia and London, is *30th April*, 1956.

Sugar Phosphates

The following chemicals of English manufacture and to the specifications shown are now available:

α-D(+)-Glucose-1-phosphoric acid, dipotassium salt dihydrate

$C_6H_{11}O_8.O.PO_3K_2.2H_2O$ Mol. wt. 372.35

Assay (ex P and K) : Not less than 95.0%
Inorganic P : Not more than 0.1% (as P)
Loss on drying : Not more than 11.0% at 105° C. in vacuo
$[\alpha]_D^{20}$ = 76° to 80° (c, 5 in water) 1 g—12s. 6d.

D(+)-Glucose-6-phosphoric acid, barium salt heptahydrate

$C_6H_{11}O_8.O.PO_3Ba.7H_2O$ Mol. wt. 521.60

Assay (ex P and Ba) : Not less than 95.0%
Inorganic P : Not more than 0.1% (as P)
Loss on drying : Not more than 25.5% at 105° C. in vacuo
$[\alpha]_D^{20}$ = 2° to 22° (c, 5 in N/1 HCl) 0.1 g—8s. 6d. 1 g—50s. 0d.

Also available:

D(+)-Fructose-1 : 6-diphosphoric acid, dibarium salt
0.1 g—4s. 3d. 1 g—25s. 0d.

D(+)-Fructose-6-phosphoric acid, barium salt
0.1 g—5s. 6d. 1 g—42s. 6d.

Enquiries for larger quantities are welcomed.

THE BRITISH DRUG HOUSES LTD.
B.D.H. LABORATORY CHEMICALS GROUP
POOLE **DORSET**
SP/5601

BIOCHEMIST

It is proposed to increase the staff of Wall's Food Laboratories at the Friary, Acton, W. 3, by the appointment of a Graduate who is interested in the biochemistry of meat.

Precise experience of our industry is not important but good qualifications are essential. There is no age range for this appointment and salary will be related to the quality of the candidate appointed.

Comprehensive superannuation and sickness benefit schemes.

Three weeks holiday a year.

Application to Staff Manager
T. WALL & SONS, LTD.
59 Park Royal Road, London, N.W. 10
(ELGAR 4871)

'ROCHE' VITAMINS

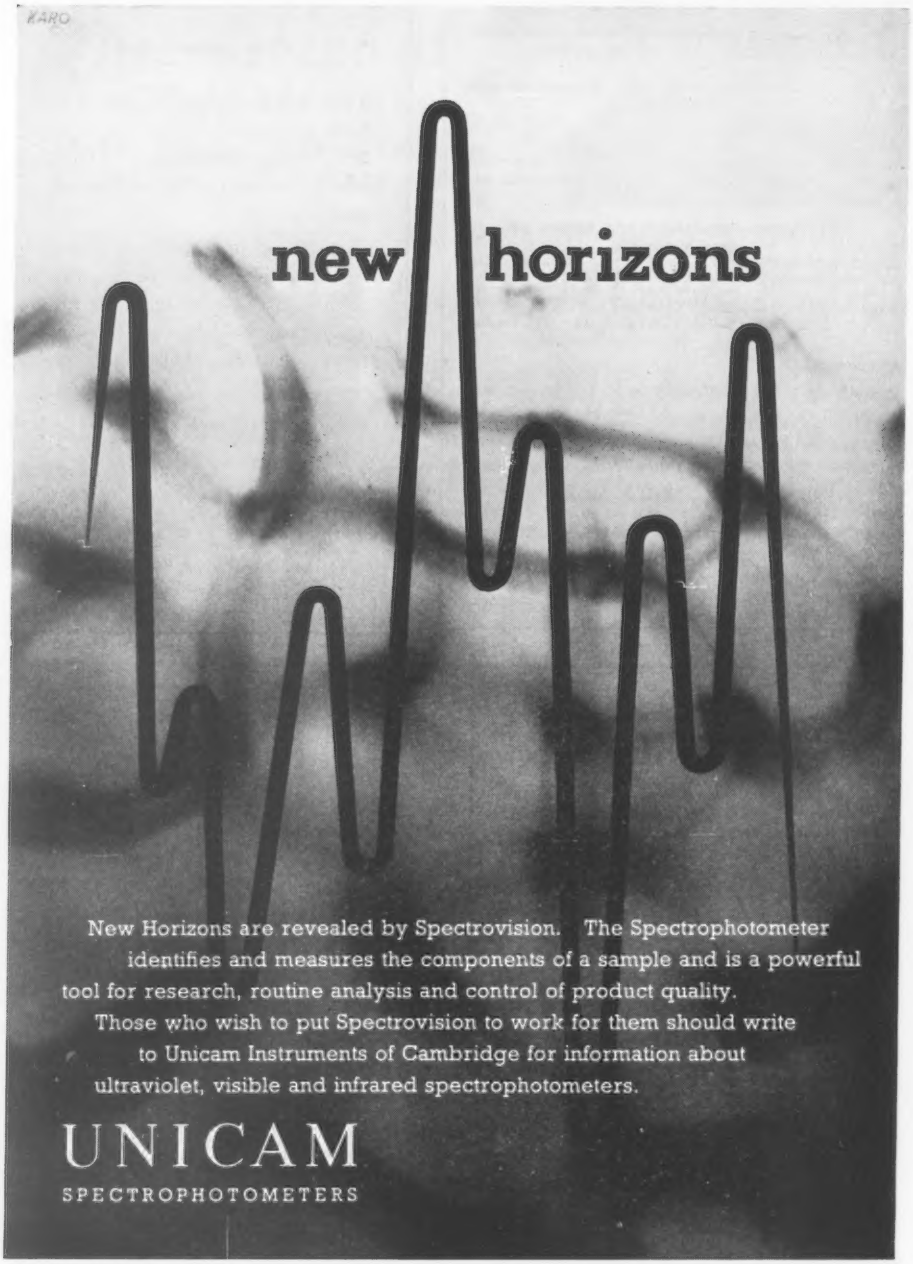
	PACKINGS (*weight in grams*)		
Vitamin A			
Vit. A acetate cryst.		1·0	
Vit. A alcohol cryst.		1·0	
Vit. A palmitate cryst.		1·0	
Vitamin B-Complex			
Aneurine hydrochloride	1·0	5·0	10·0
Co-Carboxylase	0·1		
Folic acid	1·0	10·0	
Riboflavin	0·5	1·0	10·0
Nicotinic acid	5·0	10·0	25·0
Nicotinamide	5·0	10·0	25·0
Calcium *d*-pantothenate	1·0	5·0	10·0
Pyridoxine hydrochloride	0·5	1·0	5·0
d-Biotin	0·1	0·5	1·0
p-Aminobenzoic acid	1·0	5·0	10·0
Choline chloride	5·0	10·0	25·0
Inositol	5·0	10·0	25·0
Vitamin C			
l-Ascorbic acid	1·0	10·0	25·0
Vitamin E			
Racemic *a*-Tocopherol	0·5	1·0	5·0
Racemic *a*-Tocopheryl acetate	1·0	5·0	10·0
Vitamin K			
Menaphthone	1·0	5·0	10·0

Detailed Lists on Request

ROCHE PRODUCTS LTD.
15 MANCHESTER SQUARE, LONDON, W. 1

SINGLE EXPOSURE 35MM CAMERA

Cooke Troughton & Simms
YORK ENGLAND LTD

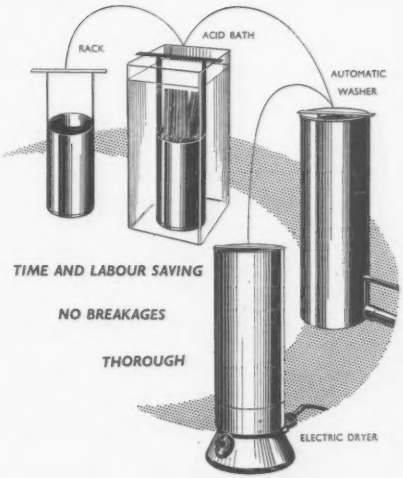

new horizons
New Horizons are revealed by Spectrovision. The Spectrophotometer identifies and measures the components of a sample and is a powerful tool for research, routine analysis and control of product quality.
Those who wish to put Spectrovision to work for them should write to Unicam Instruments of Cambridge for information about ultraviolet, visible and infrared spectrophotometers.
UNICAM
SPECTROPHOTOMETERS

Complete equipment for PIPETTE washing and drying

Here is everything necessary for overcoming the old problem of cleaning pipettes thoroughly, quickly and safely. The installation of this outfit is extremely desirable in every laboratory but becomes a necessity wherever contagious or radioactive materials are used.

The Shandon Pipette Cleaning Equipment comprises the Stainless Steel Pipette Rack, the Glass Acid Bath, Washer and the Electric Pipette Dryer. Any one item is, of course, available individually.

The pipette racks carry up to 200 pipettes of 1 ml. capacity or proportionate numbers of other sizes up to 16 in. length. Once pipettes have been put into the rack they are not handled again until removed from the dryer when they are sparklingly clean and ready for further use.



SHANDON

SHANDON SCIENTIFIC COMPANY LTD., 6 Cromwell Place, London, S.W. 1. Tel: KNightsbridge 1131

OXOID

DEHYDRATED CULTURE MEDIA

GRANULES—EASY TO HANDLE, LESS HYGROSCOPIC

TABLETS—FOR SIMPLE PREPARATION OF SMALL VOLUMES

Details upon request

Obtainable from Laboratory suppliers or direct from

OXO LIMITED (Medical Dept) THAMES HOUSE, LONDON, E.C.4.

CENtral 9781

THE LOW INERTIA WARBURG APPARATUS

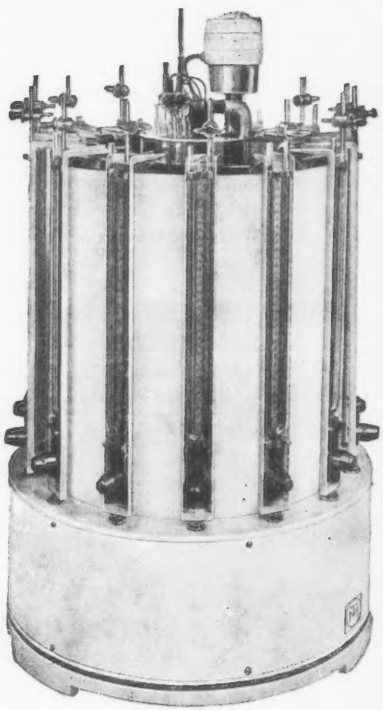
Cheaper
More compact
Silent

Takes normal manometers and flasks.

Every manometer shakes individually with a sprung semi-rotary movement and can be arrested for reading by gripping it.

Every manometer can be brought opposite the operator.

Accuracy ± 0·01°C. at 37°C.



– FOR – *"Service to Science"*

TOWNSON & MERCER LIMITED
CROYDON, ENGLAND

Telephone: THOrnton Heath 6262

THE
JOURNAL OF PHYSIOLOGY
MARCH, 1956. VOL. 131, NO. 3

BIGGERS, J. D., CLARINGBOLD, P. J. and HARDY, MARGARET H. The action of oestrogens on the vagina of the mouse in tissue culture.

DENTON, D. A. The effect of Na^+ depletion on the Na^+:K^+ ratio of the parotid saliva of the sheep.

FISHER, R. B. and LINDSAY, D. B. The action of insulin on the penetration of sugars into the perfused heart.

WHITTAM, R. The permeability of kidney cortex to chloride.

LONGSON, D., MILLS, J. N., THOMAS, S. and YATES, P. A. Handling of phosphate by the human kidney at high plasma concentrations.

HENRY, J. P. and PEARCE, J. W. The possible role of cardiac atrial stretch receptors in the induction of changes in urine flow.

BASSIR, O. Molecular inhomogeneity as a source of error in inulin clearance studies.

HODGKIN, A. L. and KEYNES, R. D. Experiments on the injection of substances into squid giant axons by means of a microsyringe.

PAASONEN, M. K. and VOGT, MARTHE. The effect of drugs on the amounts of substance P and 5-hydroxytryptamine in mammalian brain.

GASKELL, P. The significance of the 'after-drop' in venous occlusion plethysmography.

GASKELL, P. The effect of intra-arterial atropine infusions on the blood flow through the human hand and forearm.

GASKELL, P. Are there sympathetic vasodilator nerves to the vessels of the hands?

RODDIE, I. C. and SHEPHERD, J. T. The blood flow through the hand during local heating, release of sympathetic vasomotor tone by indirect heating, and a combination of both.

KATZ, B. A note on electric recording from myoneural junctions.

ANDREWS, W. H. HORNER, MAEGRAITH, B. G. and RICHARDS, T. G. The effect upon bromsulphalein extraction of the rate and distribution of blood flow in the perfused canine liver.

MCEWEN, L. M. The effect on the isolated rabbit heart of vagal stimulation and its modification by cocaine, hexamethonium and ouabain.

BORN, G. V. R. and BÜLBRING, EDITH. The movement of potassium between smooth muscle and the surrounding fluid.

BORN, G. V. R. The relation between the tension and the high-energy phosphate content of smooth muscle.

ABRAHAMS, V. C. and PICKFORD, MARY. Observations on a central antagonism between adrenaline and acetylcholine.

Proceedings.

Subscription price 80s. per volume of three parts

CAMBRIDGE UNIVERSITY PRESS
BENTLEY HOUSE, 200 EUSTON ROAD
LONDON, N.W.1

'WELLCOME'

DIAGNOSTIC AND LABORATORY MATERIALS

- AGGLUTINABLE SUSPENSIONS
- AGGLUTINATING AND PRECIPITATING SERA
- CULTURE MEDIA
- OLD TUBERCULIN
- OPACITY TUBES
- PENICILLINASE
- REAGENTS FOR COMPLEMENT FIXATION AND FLOCCULATION TESTS
- THE 'AGLA' BRAND MICROMETER SYRINGE
- SERA FOR IDENTIFICATION OF ANAEROBES

Details and Price List on application

Obtainable from: THE WELLCOME RESEARCH LABORATORIES
Langley Court, Beckenham, Kent. Telephone: Beckenham 3422 *or from:*
BURROUGHS WELLCOME & CO. *(The Wellcome Foundation Ltd.)* LONDON

E. K. BOWMAN LTD.

Established 1889

61, 61A & 63 HOLMES ROAD, KENTISH TOWN
LONDON, N.W. 5
Telephone: Gulliver 1653

★

SPECIALISTS IN
ANIMAL HOUSE EQUIPMENT
CAGES & RACKING

5102

Printed in Great Britain at the University Press, Cambridge
(Brooke Crutchley, University Printer)